PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/12, C07K 13/00, C12P 21/08, A61K 37/02, 39/395, G01N 33/68

A1

(11) International Publication Number: **WO 94/17184**

(43) International Publication Date: 4 August 1994 (04.08.94)

(21) International Application Number: PCT/US94/00517

(22) International Filing Date: 27 January 1994 (27.01.94)

(30) Priority Data:
08/010,905 29 January 1993 (29.01.93) US

(71) Applicants: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US). THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Suite 350, 900 Welch Road, Palo Alto, CA 94304-1850 (US).

(71)(72) Applicant and Inventor: PARKHOUSE, R., Michael, E. [GB/GB]; 7 BullsWater Crescent Road, Pirbright, Woking, Surrey GU2 40NF (GB).

(72) Inventors: SANTOS-ARGUMEDO, Leopoldo; Salvador Diaz Miron #344, Apartment 301, Mexico D.F. 11330 (MX). GRIMALDI, J., Christopher; 138 Parnassus Avenue, San Francisco, CA 94117 (US). BAZAN, J., Fernando; 775 University Drive, Menlo Park, CA 94025 (US). HEATH, Andrew; 135 O'Connor Street, Menlo Park, CA 94025 (US). HOWARD, Maureen, C.; 12700 Viscaino Drive, Los Altos Hills, CA 94022 (US). GOODNOW, Christopher, C.; 45 Peters Coutts Circle, Stanford, CA 94305 (US).

(74) Agents: BLASDALE, John, H., C. et al.; Schering-Plough Corporation, One Giralda Farms, M3W, Madison, NJ 07940-1000 (US).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, PT, RO, RU, SD, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MODULATION OF PHYSIOLOGICAL RESPONSES OF LYMPHOCYTES BY CD38 OR ANTIBODIES THERETO

(57) Abstract

Methods for modulating physiological responses of lymphocytes, e.g., B cells, by use of reagents based upon CD38. An enzymatic activity of the cell surface marker, or its soluble form, will be useful in modulating lymphocyte physiology, including proliferation, viability and development.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

MODULATION OF PHYSIOLOGICAL RESPONSES OF LYMPHOCYTES BY CD38 OR ANTIBODIES THERETO.

FIELD OF THE INVENTION

The present invention relates to methods of modulating a physiological response of a lymphocyte found in the immune system of an animal. More particularly, it relates to methods and compositions which have been implicated in regulation of development and/or proliferation of lymphocytes, *e.g.*, B cells.

BACKGROUND OF THE INVENTION

Vertebrates possess an active immune system which provides a surveillance mechanism that protects them from disease-causing microorganisms, *e.g.*, bacteria and viruses, and from cancer cells. The immune system specifically recognizes and selectively eliminates foreign cellular and subcellular invaders through an active process which requires constant and continuous interaction of various cell types, including T cells and B cells. Proper balance of various physiological functions between and within cells involve highly complex regulatory mechanisms which are poorly understood.

The B cell is a fundamental cell type in providing both humoral and cellular responsiveness of the immune system. The B cell is activated and differentiates in response to antigenic stimulation, but the mechanisms of these physiological processes are poorly understood. This lack of understanding prevents effective control or manipulation of the immune system in appropriate medical circumstances.

One approach to understand the regulatory mechanisms of lymphocyte function better is to study cell surface molecules using monoclonal antibodies that mimic the natural ligands. Using this strategy, several lymphocyte surface molecules have been recognized to be important in adhesion, or to act as growth factor receptors. See, *e.g.*, Clark et al., (1991) "Regulation of human B-cell activation and adhesion" *Ann. Rev. Immunol.* 9:97-127. Examples of these are: human CD23, CD40, and CD72, three antigens that have all been shown to be important in controlling the proliferation of B cells.

However, despite the ease with which the mouse system can be experimentally manipulated and explored, relatively little is known about murine B cell receptors. The lack of understanding of the regulatory mechanisms of lymphocyte physiology and development prevents effective intervention for successful treatment of medical situations where modulation of immunological function could be useful. For example, mechanisms of B cell antigen tolerance are poorly understood in spite of considerable efforts to determine the regulatory mechanisms involved therein. The present invention provides means for modulating various lymphocyte physiological responses.

SUMMARY OF THE INVENTION

The present invention is directed to the identification of surface molecules controlling activation and proliferation of B cells. Rat monoclonal antibodies were raised against antigens expressed on activated B cells. One specific antibody, NIM-R5, was characterized and found to identify a 42 kD antigen (p42). Interaction of NIM-R5 with B cells affected various physiological functions, *e.g.*, causing activation, as measured in several different assays. This implicated the surface molecule recognized by the antibody in lymphocyte physiology.

The antibody was used to isolate the cellular antigen recognized by it. Studies on the relationship of the antibody to immune function led to the discovery that the recognized antigen corresponds to a mouse CD38 counterpart. Structural analysis has suggested that the CD38 protein possesses an enzymatic activity, cADP ribosyl cyclase activity. This activity has been implicated in Ca^{2+} fluxes.

Moreover, although antibody to CD38 stimulates B cells from virtually all sources, it has been found to lack functional equivalence for particular unresponsive cell types including those with tolerance abnormalities. Thus, tolerance functions are tied in to the enzymatic activity of CD38.

The present invention provides methods of modulating a physiological response of a lymphocyte comprising contacting the lymphocyte with an antibody to CD38, a soluble fragment of CD38, or a pharmacological modulator of ADP-ribosyl cyclase, cyclic ADP-ribosyl hydrolase, or ADP-ribosyl transferase. In some embodiments, the modulating is stimulation or inhibition of lymphocyte growth or differentiation, including inhibition of growth and differentiation, and the result is establishment of antigen tolerance. Often the physiological response is mediated by a calcium flux. In other embodiments,

the lymphocyte is a B cell, and may be at a defined developmental stage, *e.g.*, one which expresses surface CD38.

In some embodiments the antibody to CD38 is polyclonal, though in others the antibody may be monoclonal, *e.g.*, NIM-R5. In others, a soluble fragment of CD38 is effective, *e.g.*, one which consists essentially of the extracellular region of CD38. The invention also embraces methods of using a pharmacological modulator which is an inhibitor of ADP-ribosyl cyclase, cyclic ADP-ribosyl hydrolase, or ADP-ribosyl transferase.

In preferred embodiments, the invention provides methods of modulating an antigen tolerance response of a B lymphocyte by contacting the lymphocyte with an antibody to CD38, a soluble fragment of CD38, or a pharmacological modulator of ADP-ribosyl cyclase, cyclic ADP-ribosyl hydrolase, or ADP-ribosyl transferase. Preferably, the modulating is inducing antigen tolerance response, *e.g.*, by using an antibody to CD38 such as NIM-R5.

The invention also provides methods of screening for a pharmacological modulator of ADP-ribosyl cyclase comprising the steps of assaying the enzymatic activity of ADP-ribosyl cyclase in the presence or absence of a candidate pharmacological modulator; and selecting a candidate which modulates said activity. Such a method will typically use a CD38 ADP-ribosyl cyclase. Typically, the candidate modulator compounds will be selected from a group of NAD analogs. The present invention also embraces pharmacological modulators selected by this method. Preferably, the pharmacological modulator will also modulate a physiological response of a lymphocyte, including a B cell.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

GENERAL OUTLINE

I. Overview
II. B cell antigen
III. NIM-R5 antibody and biological effects on B cells
IV. Biological properties of CD38
V. Structural definition of CD38
VI. Antibodies against CD38
VII. Immunology involving CD38
VIII. Therapeutic Administration

I. Overview

Various molecules are expressed on the surface of lymphocytes, including B cells, and the expression of several of these is increased upon activation: *e.g.*, Sieff et al., (1982) *Blood* 60:703-713; Paul (1993) Fundamental Immunology (3rd ed.), Raven Press, New York; and Roitt (Ed.) (1992) Encyclopedia of Immunology, Academic Press, San Diego. These molecules are good candidates for study in relation to the process of activation, differentiation, and survival of lymphocytes. Scientific inquiry has focused on the B cells as the more readily studied system, though T cell activation may be approached by similar methods. Typically, B cells are studied for their response after antigenic stimulation. Thus, B cell surface antigens are studied for their functions as growth factor receptors, and/or adhesion molecules, and are often considered in a context of their involvement in regulation of the clonal expansion and differentiation that occurs in immunized lymphatic tissues. A convenient way to explore the function of these surface molecules is through antibodies that recognize the critical epitopes and mimic the natural ligands.

Most of the latest results on B cell activation have been done on human B cells and unfortunately there are no reagents available to the corresponding murine antigens to analyze the importance of these molecules in the murine system.

A B cell antigen has been described herein by a monoclonal antibody raised against B cell surface markers expressed on LPS-stimulated cells. The screen for antibodies capable of modulating the physiological response of a lymphocyte was for markers which were specifically expressed on B cells and, more particularly, for markers whose expression was enhanced upon LPS stimulation. One resulting monoclonal antibody was the NIM-R5 described herein, which exhibited the desired properties, and immunoprecipitated a 42 kD protein, presumably a glycoprotein. The NIM-R5 was used to clone the 42 kD glycoprotein. Further analyses of the protein indicated a distant homology to human CD38, though it is probably the mouse counterpart of the human protein. Sophisticated analysis of the secondary and tertiary structural motifs of the protein suggested that it possessed an ADP-ribosyl cyclase enzymatic activity. Subsequent biochemical analysis has verified that the mouse CD38 actually possesses this activity.

Separate screening studies using the antibody showed that the NIM-R5 stimulates B cells from all tested sources except cells derived from xid mice, or "tolerant" mice from C. Goodnow. Both the xid and the "tolerant" mice share the

unusual property that they possess deficiencies in the ability to normally develop antibody responses to antigens: *e.g.*, Paul (1993) Fundamental Immunology (3nd ed.) Raven Press, New York; Goodnow (1992) *Current Opinion in Immunology* 4:703-710; and Goodnow (1992) *Ann. Rev. Immunol.* 10:489-518. This implicates the CD38 and its enzymatic activity in tolerance mechanisms.

The anti-p42 monoclonal antibody as well as anti-CD22 monoclonal antibody (NIM-R6) recently described by Torres et al., (1992) *J. Immunol.* 149:2641-2649, could help to fill the shortage of reagents necessary to study the role of these antigens in a broader physiological context. In addition to the current information already available in the murine model, it will be easier to weight the individual contribution of each factor in the whole organism by either taking the approach blocking the biological activity with antibodies or (even better) disrupting the expression of the protein using transgenic mice.

II. B cell antigens

Although B cells have been more amenable to study by these methods, a relatively low number of B cell antigens have been well characterized: *e.g.*, Barclay et al., (1992) The Leukocyte Antigen Factsbook, Academic Press, San Diego. In particular, CD23, CD40, and CD72 have been found on B cells.

CD23, an antigen present on B lymphocytes and monocytes, is upregulated upon activation. Some data in man suggest that CD23 is an autocrine growth factor important in enhancement of IL-4-induced IgE production; *e.g.*, Gordon (1991) "CD23: novel disease marker with a split personality" *Clin. Exp. Immunol.* 86:356-359. Whereas none of these activities have been demonstrated in mice, crosslinking of murine CD23 results in enhancement of class II responses, indicating partial activation.

CD24 and CD37, in contrast to the p42 antigen, are B cell specific markers (41 and 45 kD, respectively). However, their expression decreases upon activation; CD38 is a B cell associated antigen (45 kD), expressed also on T cells. No clear functions have been reported for this molecule, and to date there are no reagents available to study the mouse counterparts of CD24, CD37, or CD38.

CD40, a 48 kD antigen present on B cells, carcinomas, and follicular dendritic cells, belongs to the family of cysteine-rich receptor-like molecules that includes NGFR (Nerve Growth Factor Receptor) and TNFR (Tumor Necrosis Factor Receptor). Its expression is increased upon activation. In

addition, upon crosslinking of CD40 on B cells together with IL-4, it delivers a prolonged clonal expansion signal. Monoclonal antibodies to CD40 have also been shown to induce homotypic adhesion and enhance IL-4-induced IgE production. Its ligand has very recently been found on T cells. Monoclonal antibodies against murine CD40 have not been readily available.

CD72 is a human B cell specific antigen which was first discovered as the murine homologue Lyb 2. Polyclonal and monoclonal antibodies against Lyb 2 induce stimulation of B cells. Thus, anti-Lyb 2 induces mobilization of cytosolic-free calcium, enhancement of class II molecule expression and weak proliferation. Interestingly, anti-Lyb 2 antibodies can block the response to T-dependent antigens, but not to T-independent antigens. Recently it has been reported that CD5/Ly1 is the receptor for CD72/Lyb 2.

CD74 (41 kD) is a class II invariant chain; to our knowledge it has not been implicated in B cell activation, but there have been speculations that it may be involved in antigen processing and presentation.

Because of their functional properties, the most likely candidates to be recognized by NIM-R5 were: CD23, CD40, and CD72. Using cocapping experiments, and direct expression of these molecules on transiently transfected cells, or stable transfected cells, it was determined that NIM-R5 does not recognize any of them. Thus NIM-R5 appears to recognize a heretofore undescribed functional B cell surface marker, with a possible role in B cell activation and clonal expansion.

An important difference from other human B-cell activation antigens like CD19, CD23, and CD40 is that NIM-R5 does not costimulate the proliferation of B cells with anti-Ig antibodies. Like anti-Ig, however, NIM-R5 requires high antibody concentration and/or IL-4 for a pronounced stimulatory effect such as class II enhancement or proliferation. Unlike anti-Ig, NIM-R5 does not cause release of Ca^{2+} from intracellular stores; nor does it stimulate phosphoinositol hydrolysis.

Thus, the limited understanding of the cell surface markers expressed on B cells, along with the limited understanding of their biological functions or mechanisms of action led to the present studies on B cell surface markers.

III. NIM-R5 antibody and biological effects on B cells

A rat monoclonal antibody (NIM-R5) was prepared against a 42 kD B cell activation antigen (p42). The expression of p42 is increased upon B cell activation. NIM-R5 induces an increase of intracellular Ca^{2+}, due to influx from

the exterior milieu via calcium channels. This stimulation does not prejudice further stimulation with anti-Ig, and thus p42 constitutes an activation signal which is not identical to that mediated by membrane Ig; the activation signal mediated by membrane Ig induces release of intracellular Ca^{2+} stores.

The antibody induces increased expression of class II molecules on resting B lymphocytes and prepares the cells for "spreading" when interacted with immobilized anti-class II antibody. The antibody alone is weakly mitogenic, and comitogenic with IL-4 on resting B cells. Of particular interest, NIM-R5 induces proliferation and rescue from apoptosis in B cells activated *in vitro*. Thus, the NIM-R5 antibody induces an Ig-independent activation and proliferation of resting and activated B cells.

This antibody does not recognize other known B cell activation antigens such as CD23, CD40, or CD72. The p42 antigen may be a glycoprotein with an important role in the regulation of B lymphocyte activation and survival.

IV. Biological properties of CD38

The CD38 is not a well-studied cell surface marker. However, it has been implicated in the transduction of activation and proliferation signals in various cell types: *e.g.*, Malavasi et al., (1992) *Int'l J. Clin. Res.* 22:73-80. In particular, the marker apparently affects NK cells, B cells, and T cells.

Structurally, the human CD38 molecule has been reported to be a member of the type II integral membrane protein family. However, some soluble versions of the protein have been reported to be freely circulating in the body.

The NIM-R5 antibody was used to isolate a cDNA (I-19) encoding a B cell derived protein. This cDNA contains an open reading frame that encodes a polypeptide of 304 amino acids with a predicted molecular mass of 34,500. The existence of a 22 amino acid hydrophobic region located 23 amino acids from the amino terminal of the deduced protein, together with four potential N-linked glycosylation sites, characterize the deduced protein encoded by I-19 cDNA as a typical type II transmembrane glycoprotein. Whereas I-19 cDNA appears to encode a novel murine protein, its nucleotide sequence and deduced amino acid sequence show approximately 70% homology to the previously reported sequence of human CD38, suggesting that I-19 cDNA encodes either the mouse homologue of CD38 or a closely related protein. Northern blot analysis of the expression of this cDNA product in a variety of cell types, together with immunoprecipitation of the recombinant

protein expressed in L cells, indicated that I-19 cDNA encodes not only the epitope recognized by NIM-R5, but a protein that is indistinguishable biochemically and in terms of distribution from the murine B cell activation marker recognized by NIM-R5 antibody. Chromosomal mapping studies have localized this locus to the proximal region of mouse chromosome-5.

The anti-p42 signaling is likely to be independent of the Ig signaling pathway. From looking at biological effects such as proliferation or up-regulation of class II, NIM-R5 acts much like anti-Ig. However, anti-p42 has a completely different effect on the mobilization of calcium and does not prejudice stimulation with anti-Ig. Also, anti-p42 does not increase or modify the response induced by anti-μ or anti-δ alone, or in combination with IL-4. Another piece of evidence came from the fact that WEHI-231 and CH-31, two cell lines that express levels of p42 as high as the levels of surface IgM, can be induced into apoptosis by treatment with anti-Ig. However, anti-p42 cannot induce apoptosis or cannot rescue from the apoptosis induced by anti-Ig.

It is particularly interesting that the molecule recognized by NIM-R5 is increased on activated B cells. Other relevant and important observations are that the antibody induces an activating and proliferative signal in resting and activated B cells, rescues anti-μ chain activated B cells from apoptosis, and has a costimulatory or antagonistic effect on activated B cells with IL-4 in a time-dependent fashion.

Further studies herein on the biological functions of the CD38 protein implicate it in development of antigen tolerance.

V. Structural definition of CD38

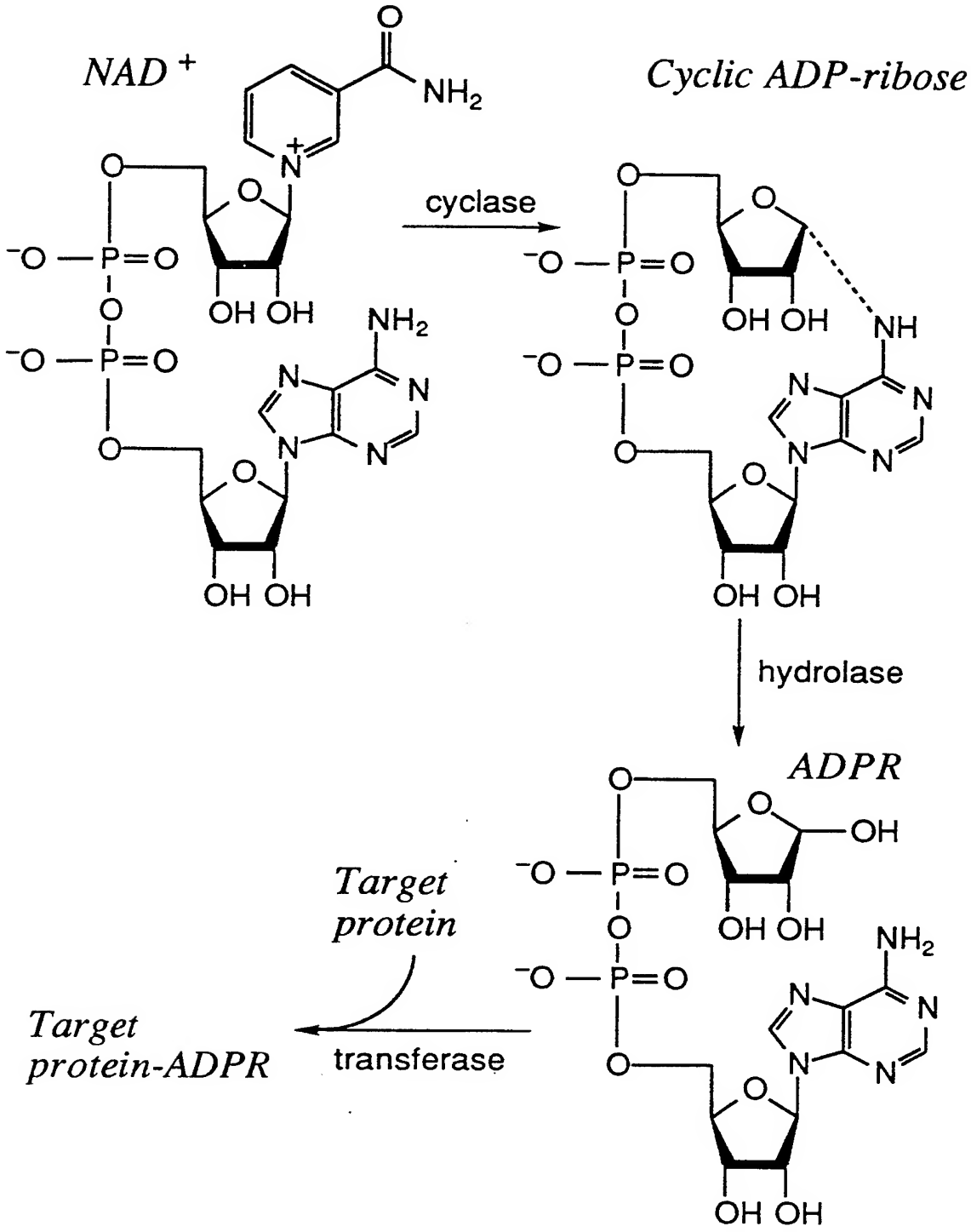
The I-19 cDNA has been characterized by sequencing. SEQ ID NOs: 1 and 2 disclose the nucleotide sequence and the derived amino acid sequence. A hydrophobicity plot of the amino acid sequence was consistent with the report that the amino terminus is close to the membrane; the carboxy proximal segment would probably provide structural and biological significance. SEQ ID NOs: 3 and 4 give respectively the nucleotide sequence encoding human CD38 and the deduced amino acid sequence for human CD38.

Structural analysis of the mouse CD38 molecule led to a hypothesis that the protein would possess an enzymatic activity corresponding to ADP-ribosyl cyclase: Lee et al., (1991) *Cell Regulation* 2:203-209. In fact, a soluble version of the CD38 molecule has been shown to possess ADP-ribosyl cyclase activity. Combining this with other observations related to Ca^{2+} flux data with NIM-R5,

has resulted in a model of the relationship of the CD38 enzymatic activity and immune function. In this model, the CD38 would function in a pathway similar to that described for similar enzymes in other systems: *e.g.*, Galione (1992) Trends in Pharmacological Sciences 13:304-306; Clapper et al., (1987) *J. Biol. Chem.* 262:9561-9568; and Clapper et al., (1985) *J. Biol. Chem.* 260:13947-13954. In particular, the enzyme typically possesses three separable activities, an ADP-ribosyl cyclase activity, a cADP-ribosyl hydrolase activity, and an ADP-ribosyl transferase activity. cADP-ribose may mediate its effects via a ryanodine receptor, suggesting additional means to find pharmacological modulators of downstream signal processes.

The ADP-ribosyl cyclase activity is a conversion of NAD into cyclic ADP-ribose and is assayed either by a calcium flux assay or by HPLC purification of enzyme reactants and products. The ADP-ribosyl hydrolase activity is a conversion of cyclic ADP-ribose into ADP-ribose, and is assayed either by HPLC purification of enzyme reactants and products or by thin-layer chromatographic analysis of enzyme reactants and products. The ADP-ribosyl transferase activity is the transfer of ADP-ribose to a specific substrate, and is assayed by mass spectrometry of substrate proteins or by radioactive label of substrate protein.

The relationships of the ADP-ribosyl cyclase, cyclic ADP-ribosyl hydrolase, and ADP-ribosyl transferase, and their substrates and products, can be shown schematically as follows, where the enzyme activities are simply denoted cyclase, hydrolase, and transferase respectively:



As used herein, the term "CD38" shall include a protein or peptide comprising amino acid sequences described in SEQ ID NO: 2 or encoded by nucleic acid sequences described in SEQ ID NO: 1, or a fragment of either entity. The term shall also be used herein to refer, when appropriate, to a

gene, or to alleles of the human or mouse component, or of other species counterparts, *e.g.*, of mammals other than humans or mice. The present invention also encompasses proteins or peptides having substantial amino acid sequence homology with the amino acid sequences in SEQ ID NO: 2.

In particular, the present invention will encompass alternative spliced variants of members of a family of related proteins having these biological or structural features.

A polypeptide "fragment", or "segment", is a stretch of amino acid residues of at least about 8 or 10 amino acids, generally at least 14 or 18 amino acids, preferably at least 22 amino acids, and, in particularly preferred embodiments, at least 26 or even 30 or more amino acids. Typically, fragments of homologous CD38 components will exhibit substantial identity.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when one regards conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: [glycine, alanine]; [valine, isoleucine, leucine]; [aspartic acid, glutamic acid]; [asparagine, glutamine]; [serine, threonine]; [lysine, arginine]; and [phenylalanine, tyrosine]. Homologous amino acid sequences are intended to include natural allelic and interspecies variations in each respective receptor sequence. Typical homologous proteins or peptides will have from 25-100% homology (where gaps can be introduced), to 50-100% homology (where conservative substitutions are included) with the amino acid sequence of SEQ ID NO: 2 or 4. Homology measures will be at least about 50% or 56%, *e.g.*, 67%or even 77%, typically at least 82% or 90%, preferably at least 93%or even 96%, and, in particularly preferred embodiments, at least 98% or more. Some homologous proteins or peptides will share various biological activities with the described proteins, *e.g.*, the embodiments provided in SEQ ID NO: 2 and 4.

VI. Antibodies against CD38

Antibodies can be raised to the various species variants of these CD38 surface antigens, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to CD38 in either their biologically or enzymatically active forms or in their inactive forms, the difference being that antibodies to the active receptor are more likely to recognize epitopes which are only present in the active protein. Anti-idiotypic antibodies are also contemplated.

Antibodies against predetermined fragments of the CD38, including binding fragments and single chain versions, can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective IL-10 receptors, or screened for agonistic or antagonistic CD38 related activity. These monoclonal antibodies will normally bind with at least a Kd of about 1 mM or less, *e.g.*, 100 or even 10 μM, generally 1 μM or even 100 nM, preferably 10nM or even 1 nM, more preferably 100 to 10 pM or less. Antibodies will be raised against species variants or other variants of these surface components.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the surface marker and inhibit ligand or substrate binding to the molecule or inhibit the ability of a ligand-like peptide or other component to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that, when the antibody binds to the receptor, the cell itself is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can bind to the marker without inhibiting ligand binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying the ligand or the enzyme itself; see, *e.g.*, Chan (Ed.) (1989) Immunoassay: A Practical Guide, Academic Press, Orlando, FL.

CD38 fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. The marker and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. For descriptions of methods of preparing polyclonal antisera, see: Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; and Williams et al., (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York. A typical method involves hyperimmunization of an animal with an antigen. Blood from the

animal is then collected shortly after repeated immunizations, and gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, cows, sheep, goats, donkeys, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, *e.g.*: Stites et al. (Eds.), Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988), Antibodies: A Laboratory Manual, CSH Press; Goding (1986), Monoclonal Antibodies: Principles and Practice (2nd ed) Academic Press, New York; and particularly in Kohler and Milstein (1975), *Nature* 256: 495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed, and cells are taken from its spleen and fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing *in vitro*. The population of hybridomas is then screened to isolate individual clones, each of which secretes a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immunized animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See Huse et al., (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," *Science* 246:1275-1281; and Ward et al., (1989) *Nature* 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, which may include the preparation of chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining to them, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include the following U.S. Patents: 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Recombinant immunoglobulins may also be produced; see Cabilly, U.S. Patent No. 4,816,567.

The antibodies of this invention can also be used for affinity chromatography in isolating the marker or in tagging cells or subcellular structures containing the marker. Columns can be prepared where the antibodies are linked to a solid support, *e.g.*, particles, such as agarose, Sephadex, or the like; a cell lysate is passed through the column, the column is washed, and then increasing concentrations of a mild denaturant are passed through, whereby the purified protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding. Highly sensitive methodologies for detection and sorting are available.

Antibodies raised against the marker will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective receptors.

VII. Immunology involving CD38

This invention provides reagents with significant therapeutic value. The CD38 proteins (naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to CD38, should be useful in the treatment of various conditions, *e.g.*, tolerance and improper physiological responses, including proliferative, viability, and developmental responses. Pharmacological modulators of the enzymatic activities should also be useful in modulating the physiological responses. In particular, the CD38, antibodies thereto, or pharmacological modulators of the enzymatic activities would be likely to have use in controlling B cell lymphomas, autoimmune situations, B cell specific proliferative abnormalities (*e.g.*, leukemias), or hypersensitivity responses.

Among the developmental responses, including differentiation processes, is establishment of antigen tolerance. The system described herein implicates Ca^{2+} fluxes in the mechanism of CD38 function. Typically, the CD38 would be expected to operate upon cells in defined developmental stages. See *e.g.* Paul (1993) Fundamental Immunology (3rd ed.), Raven Press, New York. In particular, the xid mice and the "tolerant" mice are similar in that they share inability to properly mount an antibody response against antigens. In particular, they show defects in functional response to T cell independent antigens, although the tolerant mice are also defective in

functional responses to T cell dependent antigens. Thus, the processes involved in responding to the appropriate T independent responses are suggested to involve CD38, *e.g.*, the enzymatic activity and its Ca^{2+} pathway. See *e.g.* Paul (1993), Fundamental Immunology, Raven Press, New York.

VIII. Therapeutic Administration

Additionally, this invention should have therapeutic value in any disease or disorder associated with abnormal expression or abnormal triggering of CD38. For example, it is believed that CD38 plays a role in many basic regulatory processes in immune function. Agonists and antagonists of the surface marker will be developed using the present invention.

Recombinant CD38 itself or antibodies to CD38 can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, *e.g.*, in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous stabilizers and excipients; see Berkow (Ed.), The Merck Manual, Merck, Rahway, NJ. These combinations can be filtered sterile and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof which are not complement-binding.

Drug screening using the CD38 or antibody which recognizes it, or fragments thereof, can be performed to identify compounds having binding affinity to the marker or ligands binding to it. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks an activity of a CD38-related component, *e.g.*, a binding compound. Likewise, a compound having intrinsic stimulating activity can activate the marker and is thus an agonist in that it stimulates an activity of the enzyme. This invention further contemplates the therapeutic use of antibodies to CD38 as antagonists. Pharmacological modulators of the enzymatic activity will also find use.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicaments administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of

human dosage. Various considerations are described, *e.g.*, in Gilman et al. (Eds.), (1990) Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, Tarrytown, NY, and in Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, *e.g.*, for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, *e.g.*, in The Merck Index, Merck & Co., Rahway, New Jersey. See also (*e.g.*) Avis et al. (Eds.), (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Dekker, NY, and Leiberman et al. (Eds.), (1990) Pharmaceutical Dosage Forms: Disperse Systems, Dekker, NY. Low dosages of these reagents would be initially expected to be effective. Thus, dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 100 fM (femtomolar), with an appropriate carrier. Slow-release formulations or slow-release apparatus will often be utilized for continuous administration.

The CD38, fragments thereof (including extracellular segments), and antibodies to this marker or its fragments, antagonists, and agonists, may be administered directly to the patient, however, depending on the size of these compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. Whereas it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers therefor. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral administration (including subcutaneous, intramuscular, intravenous and intradermal administration). The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy: *e.g.*, Gilman et al. (Eds.), (1990) Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press; and Remington's Pharmaceutical

Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Screening using p42 for binding partners or compounds having binding affinity to p42 antigen can be performed, including isolation of associated components. Subsequent biological assays can then be used to determine if the compound has intrinsic biological activity and is therefore an agonist or antagonist in that it blocks an activity of the antigen. This invention further contemplates the therapeutic use of antibodies to p42 protein as antagonists. This approach should be particularly useful with other p42 protein species variants and other members of the family.

The critical structural elements that effect various physiological or differentiation functions can be dissected with standard techniques of modern molecular biology, especially in comparing members of the related family; see (*e.g.*) the homolog-scanning mutagenesis technique described by Cunningham et al., (1989) *Science* 243: 1339-1336, and approaches used by O'Dowd et al., (1988) *J. Biol. Chem.* 263: 15985-15992 and by Lechleiter et al., (1990) *EMBO J.* 9:4381-4390. The invention also provides means, *e.g.* chemical cross-linking and immunoprecipitation, to isolate other proteins that specifically interact with p42, *e.g.* the intracellular domain.

In particular, functional domains or segments can be substituted between species variants or related proteins to determine what structural features are important in both binding partner affinity and specificity, as well as signal transduction. Cell markers may mediate their effects through interactions involving multiprotein complexes: *e.g.* p42 might be found as one member of a multiprotein membrane complex. An array of different variants will be useful to screen for molecules exhibiting various combinations of properties, *e.g.*, interaction with different species variants.

The broad scope of this invention is best understood with reference to the following Examples, which are not intended to limit the invention in any manner.

EXAMPLES

EXAMPLE 1:

Methods

Generally, standard methods were used with minor modifications. In particular see: Coligan et al. (Eds.), (1991 and periodic supplements) Current Protocols in Immunology, Greene/Wiley, New York; Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York; Ausubel et al., (1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Deutscher (Ed.), "Guide to Protein Purification" from Methods in Enzymology vol. 182, Academic Press, San Diego; and other standard references for laboratory techniques. See also: Santos-Argumedo et al., (1993) *J. Immunol.* 151:3119-3130; Harada et al., (1993) *J. Immunol.* 151:3111-3118; and Howard et al., (1993) *Science* 262:1056-1059.

Mice

(CBA x C57) F_1 mice were produced in the National Institute for Medical Research animal facility and used at 6-8 weeks of age.

Medium

RPMI-1640 (Flow Labs) was supplemented with non-essential amino acids (Gibco), 5 X 10^{-5} M 2-mercaptoethanol (Sigma), 1 mM sodium pyruvate (Sigma), 2 mM glutamine (Sigma) and 5% (v/v) Fetal Bovine Serum (NBL).

Fresh B cell isolation

Fresh B cells were isolated from spleen using anti-Thy-1 monoclonal antibody ascites (NIM-R1; Chayen et al., (1982) *J. Immunol. Methods*, 49:17-23) plus idubiose A37 (IBF Biotechnics)-absorbed guinea pig complement to kill T lymphocytes, followed by separation on Percoll™ (Pharmacia) to purify the small resting B cells. The population with $\rho > 1.080$ was > 90% sIg^+. [Percoll™ is a density-gradient medium consisting of a sterile solution of silica particles (15 to 30 nm in diameter) coated with non-dialyzable polyvinylpyrrolidone.]

Cell Culture

Cells were cultured at 0.5-1 x 10^6 cells/ml. For analysis of [^{3}H]-thymidine uptake, cultures of 10^5 cells in 200 µl in flat-bottom tissue culture plates were labeled for 4 hours with 0.5 µCi of [^{3}H]-thymidine before being harvested and counted.

Several murine pre-B or B cell lines (WEHI 231, A20, CH12, CH31), and the murine EL4 thymoma, were obtained from the American Type Culture Collection (Rockville, MD). Antigen-activated D10 T helper clone cells were kindly provided by Dr. Anne O'Garra (DNAX) and were prepared as described by Kaye et al., (1983) *J. Exp. Med.*, 158:836-856. Mouse livers were obtained from 8-week-old female BALB/c or C57BL/6 mice purchased from Simonsen Laboratories (Gilroy, CA). The CCE embryonic stem cell line derived from 129/Sv mice was obtained from Dr. Werner Muller (University of Cologne).

Preparation and characterization of monoclonal antibodies

BCL_1 lymphoma B cell plasma membranes were prepared as descibed by Snary et al. (1976) *J. Analyt. Biochem.* 74:457-465, dissolved in 2% (w/v) sodium deoxycholate - 50 mM Tris HCl - 50 mM NaCl, pH 8.3 (DOC-Tris), and then passed over a column of Lentil lectin-Sepharose (Pharmacia), equilibrated with DOC-Tris. The absorbed lymphocyte plasma membrane glycoproteins were eluted with 0.1 M α-methylmannoside-DOC-Tris, dialyzed, and injected into footpads of Lou strain rats (Kearney et al., (1981) *Eur. J. Immunol.*, 11:877-883). The resulting immune popliteal lymph node cells were fused with the J.K. mouse myeloma cell line (see Kearney et al., (1979) *J. Immunol.*, 123:1548-1550), and antibodies were selected by their positive reactions with splenic B cells. NIM-R6 recognized murine CD22 (Torres et al., (1992) J. Immunol. 149:2641-2649). NIM-R7 recognized a 58 kD surface molecule on BCL_1 cells. NIM-R8 recognized a 90 kD molecule on the surface of both B and T lymphocytes. NIM-R9 and NIM-R10 recognized murine IgD and IgM via δ-chain and µ-chain determinants, respectively.

Lactoperoxidase-catalyzed surface iodination of splenic (CBA x C57)F_1 B cells and immune coprecipitation and SDS-PAGE were performed by standard procedures as described in Abney et al., (1976) *Nature*, 259:404-406.

Splenic lymphocytes (T and B, and purified B) were activated with lipopolysaccharide (LPS) (50 µg/ml) or concanavalin A (Con A) (1 µg/ml) in medium. Resting and activated cells were stained with the rat NIM-R5 monoclonal antibody followed by specifically absorbed goat anti-rat Ig-phycoerythrin (PE) (Southern Biologicals, Birmingham, Alabama, U.S.A.)

and then counterstained, either with specifically absorbed goat anti-mouse Ig-fluorescein (FITC) (Southern Biologicals, Birmingham, Alabama, U.S.A.) or with a rabbit anti-purified Thy-1 antigen-FITC (generously provided by Dr. Alan Williams, Oxford University, England). The cells were then analyzed in a Beckton-Dickinson FACScan machine with the appropriate settings for small dense lymphocytes or the larger activated cells.

NIM-R5 rat IgG_{2a} antibody, or B3B4 anti-mouse CD23 rat IgG_{2a} antibody (Pharmingen, San Diego, CA) as an isotype control, was purified from serum-free hybridoma supernatants by HPLC as described: Nau, (1987) "ANx: A novel chromatographic matrix for the purification of antibodies" in Commercial Production of Monoclonal Antibodies: A Guide for Scaling-Up Antibody Production, Seaver (Ed.), Marcel Dekker, New York, pp. 247-275. For immunofluorescence studies, these antibodies were biotinylated by standard procedures, and used in conjunction with phycoerythrin-conjugated streptavidin (Becton-Dickinson, Mountain View, CA).

Monoclonal antibody purification

Monoclonal antibodies were raised *in vitro* in tissue culture medium. Each sample was dialyzed extensively with 10 mM 2-[N-morpholino]ethane-sulfonic acid (MES) (Sigma) pH 5.6 and then loaded at 0.5 ml/minute into a Bakerbond ABx gold column (7.75 mm x 10 cm) (J.T. Baker). After 10 minutes in 10 mM MES, the sample was eluted with a continuous linear gradient from 0 to 50% (v/v) with 1M sodium acetate (Sigma) pH 7.0, for 40 min at 0.5 ml/minute.

Class II antigen analysis

Cells in phosphate-buffered saline (PBS), 1% (w/v) Bovine Serum Albumin (Sigma), and 0.2% (w/v) sodium azide were stained with FITC-NIM-R4 monoclonal antibody (Andrew et al., (1985) Immunology, 54:233-240) for 30 minutes at room temperature, then washed three times and fixed with 1% (w/v) formaldehyde in PBS. Quantitative fluorescence analysis was performed using FACS. The cells were selected on the basis of forward scattering and side scattering of the light.

Spreading (Morphological transformation)

For spreading (Cambier et al., (1989) *J. Exp. Med.*, 170:877-886), polystyrene 24-well-plates were coated with 1 ml of different monoclonal antibodies at 20 µg/ml in PBS (4 hours' incubation at 37°C or overnight incubation at 4°C). The plates were "blocked" with PBS-10% (v/v) Fetal Bovine Serum by incubation at 37°C for 1 hour; they were then washed with medium

extensively before use. The uncoated plates were treated as the others but without antibody. For priming, 10^6 small dense B cells were incubated with monoclonal antibodies (B7.6 anti-mouse μ chain, or NIM-R5) at 10 μg/ml plus 10 U/ml of IL-4 (gift from DNAX Research Institute, Palo Alto, CA) for 18 hours at 37°C, then transferred with the stimuli to the precoated plates and incubated at 37°C for 1 or 16 hours. The observation was done using an inverted microscope with 100X or 200X magnification.

Measurement of intracellular Ca^{2+}

5×10^7 small dense B cells in medium were loaded with 2 μM INDO-1 AM (Molecular Probes) by incubation at 37°C for 30 min. To inhibit the release of the internal stores of Ca^{2+}, the cells were first loaded with 50 μM of BAPTA-AM (Molecular Probes) in medium, incubated at 37°C for 30 min, and then without washing or changing the medium, INDO1-AM was added as described. After loading, cells were washed three times with Hank's solution plus 10 mM Hepes pH 7.3 and adjusted to 10^7 cells/ml. Ca^{2+} was measured in a Perkin-Elmer thermally jacketed fluorescence spectrophotometer MPF-4. Before measuring, the thermal jacket was adjusted to 37°C and the fluorescence spectrophotometer was set up to 340 nm for excitation and 390 nm for emission. For the analysis, 810 μl of the cell suspension was added to the cuvette and the baseline was allowed to equilibrate; then 90 μl of the stimulus was added and the fluorescence (F) was recorded for 10 minutes. In experiments that involved the depletion of external Ca^{2+}, 50 μl of 0.2 M EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid] (Sigma) in PBS was added to the cells and the baseline was allowed to equilibrate before the stimulus was added. Fluorescence maxima (Fmax) was measured by adding 50 μl of 0.2 mM diethylenetriaminepentaacetic acid (DTPA) (Sigma) plus 1% (v/v) Triton X-100 in PBS; Fluorescence minima (Fmin) were measured by the addition of 50 μl of 0.2 M EGTA (Sigma) in PBS, and 100 μl of 0.44 M Tris (tris[hydroxymethyl]amino-methane) (Sigma) in water. The concentration of intracellular Ca^{2+} was calculated with the following formula for INDO-1:

$$[Ca^{2+}]i = Kd\left(\frac{F-Fmin}{Fmax-F}\right), \text{ where } Kd = 250$$

Preparation of activated B cell blasts

Purified small dense B cells (10^6/ml) were cultured with 50 μg/ml of LPS from *E. coli* serotype 055:B5 (Sigma) or with 20 μg/ml of B7.6 monoclonal

antibody to mouse μ-chain plus 10 U/ml of IL-4. After 72 hours of incubation at 37°C, the cells were harvested, washed with PBS, and purified in two-step Percoll™ gradients at 50% (v/v) and 70% (v/v) in PBS. The cell suspension was applied to the top of the gradient and centrifuged at 1000 x g for 15 min. The B cell blasts were taken from the interface 50%-70%, washed, and adjusted in medium to 10^6 cells/ml.

Purification and analysis of total DNA

10^7 cells were lysed in 2 ml of lysis buffer containing 1% (w/v) SDS (Sigma), 0.2 mg/ml Proteinase K (Boehringer Mannheim), 0.1 M NaCl, 10 mM Tris-HCl, 1mM EDTA, pH 8.0, and 100 μg/ml Ribonuclease Type 1-AS (Sigma). The mixture was incubated for 3 hours at 50°C and the resulting lysate extracted with 1:1 phenol-chloroform. The two phases were mixed and then separated by centrifugation at 4°C. Genomic DNA was precipitated overnight at -20°C with 0.3 M sodium acetate and two volumes 100% (v/v) ethanol. DNA was sedimented by centrifugation and the DNA pellet was air-dried and redissolved in 0.2 ml TE buffer pH 8.0 (10 mM Tris-HCl, 1 mM EDTA). DNA samples were loaded at 2 μg/track on a 1.5% (w/v) agarose gel containing 1 μg/ml ethidium bromide. DNA was visualized under UV light.

cDNA Library Construction

Poly(A)+ RNA isolated from a murine pre-B cell line, WEHI 231, was converted to double-stranded cDNA using cDNA synthesis system (Promega, Madison, WI). *Bst*XI linkers (Invitrogen, San Diego, CA) were attached, and cDNA larger than 850 bp was isolated by agarose gel electrophoresis. The size-selected cDNA was inserted into the *Bst*XI sites of the pME18S vector (a kind gift of K. Maruyama and A. Miyajima; DNAX), a derivative of the pCEV4 cDNA expression vector. See Itoh et al., (1990) *Science* 247:324-327. Approximately 1.46 x 10^6 independent clones were obtained.

Screening of cDNA Library

cDNA clones encoding the antigen recognized by NIM-R5 antibody were isolated using a modification of the method of Seed and Aruffo, (1987) *Proc. Nat'l Acad. Sci. USA*, 84:3365-3369. COS7 cells (8 x 10^6 cells in serum-free DMEM) were transfected by electroporation (200V, 960 mF, using Bio-Rad Genepulser) with 20 μg of cDNA plasmid. After 3 days' culture, cells were harvested into PBS containing 5 mM EDTA and panned on tissue culture plates coated with NIM-R5 antibody. Unbound COS7 cells were washed away, and cDNA was extracted from COS7 cells bound to the plates and transformed into *E. coli* by electroporation in order to amplify recovered plasmids. Specific

plasmids were enriched by four sequential pannings, then evaluated individually for NIM-R5 epitope expression by transient transfection into COS7 cells and immunofluorescence analysis.

Immunofluorescence

Transiently transfected COS7 cells or stably transfected L cells were stained with biotinylated NIM-R5 or isotype control antibodies added to cell pellets at 10 mg/ml. Following washing, cells were further incubated with phycoerythrin-conjugated streptavidin, then analyzed using a FACScan. Dead cells were excluded on the basis of forward angle and side scatter.

Stable Transfectants

cDNA clones were inserted into the pME18S vector containing the Neomycin resistance gene, and 20 µg of linearized plasmid were then transfected into L cells using the calcium phosphate precipitation method; Kingston, (1987) "Calcium phosphate transfection" in Current Protocols in Molecular Biology, Ausubel (Ed.), John Wiley & Sons, New York. Transfected cells were cultured for 48 hr, and then selected in medium containing G418 (0.5 mg/ml). G418 resistant L cells were selected for intensity of NIM-R5 expression by flow cytometry.

Nucleotide Sequence Analysis

Nucleotide sequencing was carried out with minipreparations of dsDNA using the dideoxy chain termination method using Sequenase Version 2.0, U.S. Biochemicals, Cleveland, OH. The DNA sequence reported is based on sequencing both strands. Sequence data were compiled and analyzed using the Intelligenetics Suite program (Intelligenetics, Mountain View, CA) and GCG program (Genetics Computer Group, Inc., Madison, WI). Database searches were carried out with the Intelligenetics program "FASTDB" and GenBank release #71. The DNA sequence reported has been submitted to GenBank under accession number L113320.

Immunoprecipitation

Transfected and untransfected L cells were surface-radioiodinated using the lactoperoxidase-catalyzed reaction (Sigma, St. Louis, MO), and were subsequently lysed with 0.5% NP-40 (Pierce, Rockford, IL). The lysates were immunoprecipitated with NIM-R5 antibody or an irrelevant rat IgG2a (Zymed, San Francisco, CA) as isotype control, followed by the addition of goat anti-rat IgG-Sepharose (Zymed). Immunoprecipitates were then analyzed by SDS-PAGE.

Northern Blot Hybridization Analysis

Poly(A)$^+$ RNA was isolated from CH31, CH12, WEHI 231, A20, EL-4, and D10 cell lines using FastTrack system (Invitrogen, San Diego, CA). Two micrograms of poly(A)$^+$ RNA were applied to agarose gel electrophoresis in the presence of 2.2 M formaldehyde, transferred to a nylon filter, and hybridized with ^{32}P-dCTP-labeled I-19 clone insert.

Interspecific Mouse Backcross Mapping

Interspecific backcross progeny were generated by mating (C57BL/6J x M. spretus) F_1 females and C57BL/6J males; Copeland et al., (1991) *Trends Genet.* 7:113-118. A total of 205 N_2 progeny were obtained; a random subset of these N_2 mice was used to map the Cd38 related sequence (Cd38-rs) locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described by Jenkins et al., (1982) *J. Virol.*, 43,26-36. Blots were prepared with Zetabind nylon membrane (AMF-Cuno). A mouse Cd38-rs cDNA clone (pME18S) was labeled with [α-^{32}P]-dCTP using a nick-translation labeling kit (Boehringer Mannheim); washing was done to a final stringency of 0.2 X SSCP, 0.1% SDS, 65°C. Fragments of 9.4, 7.5, and 5.1 kb were detected in *Pst*I-digested C57BL/6J DNA, and fragments of 8.4, 5.6, 5.1, 2.6, and 1.2 kb were detected in *Pst*I-digested M. spretus DNA. The presence or absence of the 8.4, 5.6, 2.6, and 1.2 kb M. spretus-specific *Pst*I fragments, which cosegregated, was followed in backcross mice.

The probes and RFLPs for the loci linked to Cd38-rs including interleukin-6 (IL-6), homeo box - 7.1 (Hox-7.1) and the kit proto-oncogene (Kit) have been described by Hill et al., (1989) *Genes and Dev.*, 3:26-37. Recombination distances were calculated as described by Green (1981) "Lineage, recombination and mapping" in Genetics and Probability in Animal Breeding Experiments, Oxford University Press, New York, pp. 77-113, using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

EXAMPLE 2: Characterization of the antigen recognized by NIM-R5 monoclonal antibody

This Example investigated the distribution of p42 on murine T and B cells, and immunoprecipitation of the antigen recognized by NIM-R5 monoclonal antibody.

Splenic small dense cells were stained with a combination of monoclonal antibody and PE-goat anti-rat Ig (specifically absorbed with mouse Ig (Southern Biologicals)) and then counterstained with rabbit anti-Thy 1-FITC or goat anti-mouse Ig-FITC (specifically absorbed with rat Ig (Southern Biologicals)). The cells were analyzed in a FACScan with the appropriate settings for lymphocytes. The surfaces of splenic (CBA X C57)F_1 B cells were labeled with ^{125}I using lactoperoxidase, immunoprecipitated with the monoclonal antibody NIM-R5 and then analyzed in SDS-PAGE using standard procedures.

The reactivity of monoclonal antibody NIM-R5 was characterized by FACS analysis of splenic small dense lymphocytes stained with a combination of monoclonal antibody and PE-goat anti-rat Ig, and then counterstained with FITC-rabbit anti-Thy 1 or FITC-Goat anti-mouse Ig-FITC (specifically absorbed with rat Ig (Southern Biologicals)). The cells were analyzed in a FACScan with the appropriate settings for lymphocytes. NIM-R5 stained mainly Thy 1 negative cells; however staining of a few Thy 1+ cells could not be excluded. Some other cells were Thy 1-, NIM-R5. All sIg+ cells were NIM-R5 positive; at most only very few sIg- cells were positive for NIM-R5, and even then the staining was not as bright as the staining of the Ig+ cells. The results suggest that NIM-R5 recognizes a determinant expressed mainly on B cells, although perhaps on a small population of Thy 1+ cells. The Thy 1-, sIg- cells are also negative for the expression of antigen recognized by NIM-R5. Splenic (CBA X C57)F_1 B cells were labeled on their surfaces with ^{125}I using lactoperoxidase, immunoprecipitated with the monoclonal antibody NIM-R5 and then analyzed in SDS-PAGE using standard procedures showed recognition by NIM-R5 of a 42 kD protein.

Activated B cells expressed more of the NIM-R5 determinant p42 than resting B cells. T cell depleted small dense splenic B cells were activated *in vitro* with LPS (50 µg/ml) and then harvested at day 1, 2, and 3, purified through Percoll™, stained with NIM-R5-FITC and then analyzed in FACScan with the appropriate settings for small cells and large activated B blasts. For day 0, fresh small dense B cells were analyzed. The mean intensity of the fluorescence increased steadily from day 0 (119) through day 3 (562). Analysis of median fluorescence intensities of the cells showed that expression of p42 is upregulated after activation. *In vivo* activated large B cells also show higher expression of p42 (mean fluorescence intensity = 379) when compared with small resting B cells; in contrast, Con-A activated T cells did not express

the antigen defined by NIM-R5. With the exception of EL-4 cells, most T cell lymphomas were also negative, whereas the majority of B cell lines were positive (Table 1).

Table 1: Distribution of p42 antigen on murine lymphomas.

Name	Description	Mean Fluorescence Intensity	
		Control	NIM-R5
BaF/3	B-cell precursor (Pro-B)	7.16	7.70
70Z/1.3	B cell precursor (Pre-B)	4.69	14.42
CH-31	Immature B cell	4.37	35.93
CH-33	Immature B cell	3.62	34.82
WEHI-231	Immature B cell	3.93	59.15
BCL_1	B activated cell	5.34	62.05
A-20	Differentiated B cell	5.90	8.41
Ag.8 (J.K.)	Plasmacytoma	10.08	12.14
SP/2	Plasmacytoma	11.45	13.38
LBRM33 1A5	T-cell precursor	3.12	3.45
BWS147	Immature T cell	5.66	7.35
EL-4	Immature T cell	5.57	46.50
C6VL	Mature T cell	9.61	10.33
CTLL-2	Mature T cell	7.65	8.04
H-T2	Mature T cell	4.46	5.14

Taken together, these results suggest that NIM-R5 recognizes a B cell specific antigen which is upregulated upon activation.

EXAMPLE 3: NIM-R5 monoclonal antibody induces an Ig-independent increase of intracellular Ca^{2+}.

NDO-1AM loaded B cells stimulated with anti-μ (B7.6), anti-δ (NIM-R9), or different concentrations of anti-p42 (NIM-R5) monoclonal antibodies were analyzed for increased concentration of cytoplasmic calcium using fluorescence spectrophotometry. (The concentration of intracellular calcium was calculated as described above.) Anti-Ig antibodies have been shown to increase the concentration of intracellular Ca^{2+} in B cells. It was observed that NIM-R5 monoclonal antibody could also increase the intracellular concentration of Ca^{2+}, though with completely different kinetics from those of anti-μ and anti-δ antibodies. Anti-μ and anti-δ antibodies caused an early, very

rapidly increasing concentration followed by a decrease to steady levels; these kinetics have been explained by the release of Ca^{2+} from internal stores of Ca^{2+} during the first two minutes followed by opening of surface membrane Ca^{2+} channels. However, on stimulation with NIM-R5, the first phase is missing. This suggests that NIM-R5 does not induce release from internal stores but does induce the opening of membrane Ca^{2+} channels to the exterior. Moreover, the increased cytoplasmic Ca^{2+} levels then remained constant over 10 minutes. NIM-R5 caused a 10% increase at 50 μg, a 5% increase at 25 μg, and no increase at 10 μg.

To test the hypothesis that NIM-R5 does not induce release from internal stores but does induce the opening of membrane Ca^{2+} channels to the exterior, both calcium influx in BAPTA-AM treated B cells and EGTA inhibition of calcium influx induced by monoclonal antibody anti-p42 (NIM-R5) were tested. BAPTA-AM inhibits Ca^{2+} release from internal stores without affecting the influx of external Ca^{2+} via Ca^{2+} channels. BAPTA-AM-treated B cells loaded with INDO-1AM were stimulated with anti-μ or anti-p42 monoclonal antibodies and analyzed for an increase in concentration of cytoplasmic calcium by fluorescence spectrophotometry as above. INDO-1AM loaded B cells were analyzed by fluorescence spectrophotometry. BAPTA-AM-treated B cells failed to exhibit the initial rapid increase of intracellular Ca^{2+} when stimulated with anti-μ chain or anti-δ chain, but continued to respond to NIM-R5, giving an increase of intracellular Ca^{2+}, presumably due to influx via calcium channels. The same later-sustained elevated Ca^{2+} response was also obtained with anti-Ig reagents. A similar conclusion was reached by demonstrating inhibition of NIM-R5-mediated Ca^{2+} flux by removing the external source of Ca^{2+}; EGTA completely abrogated the influx of Ca^{2+} upon stimulation with NIM-R5. However, further stimulation of the same cells with anti-μ induced the early release of Ca^{2+} from internal stores. Taking these results as a whole, NIM-R5 increases intracellular Ca^{2+} through the influx from the exterior milieu, but does not induce the release from internal stores, and also does not cause desensitization of the response induced by anti-μ, as does anti-δ chain antibody.

EXAMPLE 4: <u>NIM-R5 induces increase of expression of Class II molecules on resting B cells and prepares the cells for spreading</u>

One of the early steps of activation of small dense B cells is related to the enhancement of expression of class II molecules as well as the increase of other molecules needed for the interaction of the B cells with T and other

accessory cells. IL-4 induces the highest increase in class II molecules and seems to be the only interleukin to induce this phenomenon on murine small resting B cells. Purified small dense resting B cells were incubated with IL-4 (10 units/ml) or NIM-R5 (50 µg/ml) for 16 hours, harvested, stained with anti class II monoclonal antibody (NIM-R4-FITC), and then analyzed in a Becton-Dickinson cell sorter. Incubation with IL-4 or NIM-R5 resulted in increased mean intensities of FITC-anti-class II FACS staining from 517 to 596 and to 577 respectively. This upregulation is specific because other monoclonal antibodies against B cell surface markers, with the same isotype, do not induce this enhancement. This enhancement is titratable and requires at least 50 µg/ml of monoclonal antibody to induce enhancement on approximately 50% of the cells.

Simultaneously with the upregulation of class II molecules, there are several other changes that prepare the B cells to interact with other cells in order to receive the appropriate signals for proliferation and differentiation. Cambier's group described the morphological transformation of small resting B cells primed with anti-µ and IL-4 and then incubated in plates precoated with anti-class II monoclonal antibodies. In this experimental design, NIM-R5 plus IL-4 primed small resting B cells for spreading and for morphological transformation on anti-class II precoated plates. The priming is similar to that obtained with either B7.6 anti-µ or NIM-R9 anti-δ monoclonal antibodies. Both require incubation for 18 hours with 10 µg/ml of monoclonal antibody and 10 U/ml of IL-4. Anti-µ or NIM-R5 by itself primes only a small percentage of B cells for spreading (< 20%). IL-4 alone induces spreading in a slightly higher percentage (20-30%). However, a mixture of B7.6 or NIM-R5 with IL-4 induces spreading in more than 70% of the cells. At the concentrations used in these experiments, there was no difference in priming between B7.6 anti-µ monoclonal antibody or NIM-R5; neither B7.6 nor NIM-R5 could be substituted for NIM-R4 anti-class II monoclonal antibody as inducers of the spreading process in the second phase of the assay. After only one hour of incubation, the primed cells start to show dendritic processes on anti-class II precoated plates; however, the length and number of dendritic prolongations reached a maximum after 18 hours of incubation at 37°C.

EXAMPLE 5: <u>NIM-R5 is weakly mitogenic, and comitogenic with IL-4 on small resting B cells</u>

NIM-R5 induces proliferation of small resting B cells. Compared with other mitogenic antibodies like B7.6 (anti-µ) or NIM-R9 (anti-δ), NIM-R5 has the

same dose-response profile of mitogenicity. In this experiment, small dense B cells were cultured with different concentrations of monoclonal antibodies and different concentrations of IL-4, as indicated. After 72 hours of incubation, the cells were pulse-labeled with ^{3}H-thymidine for 4 hours and harvested, and the incorporated ^{3}H-thymidine was measured. The addition of even 1 U/ml of IL-4 causes a significant increase in the mitogenicity of the monoclonal antibodies. Proliferation was maximal with the addition of 10 U/ml of IL-4, and further addition of interleukin did not enhance proliferation. In contrast, other monoclonal antibodies against B cell surface markers like NIM-R10 (a non-mitogenic anti-μ), or NIM-R6 (anti-CD22), NIM-R7 (anti-p58) or NIM-R8 (anti-p90) were unable to induce proliferation on their own or in the presence of IL-4.

Anti-p42 monoclonal antibody failed to costimulate B cells activated with anti-Ig or anti-Ig plus IL-4. Small dense B cells were cultured with different combinations of monoclonal antibodies without IL-4 or with IL-4. After 72 hours' incubation, the cells were pulsed with ^{3}H-thymidine for 4 hours and harvested, and the incorporated ^{3}H-thymidine was measured.

The proliferation induced by NIM-R5 on its own was small and reached maximum levels at 50 μg/ml correlating with the induction of class II molecules. This was similar to the poor stimulatory effect that either B7.6 (anti-μ chain) or NIM-R9 (anti-δ chain) has on its own. Comparing the same concentrations of antibody and IL-4, NIM-R5 did not induce as high proliferation as anti-μ chain or anti-δ chain. However, there was no costimulatory effect between NIM-R5 and anti-μ chain or anti-δ chain with or without IL-4; neither was there an antagonistic effect between these two signals because NIM-R5 did not inhibit proliferation induced by monoclonal anti-μ chain or anti-δ chain.

EXAMPLE 6: <u>NIM-R5 induces proliferation and rescue from apoptosis on anti-μ plus IL-4 activated B cells</u>

B cell blasts activated for three days with anti-μ monoclonal antibody B7.6 (10 μg/ml) and IL-4 (10 units/ml), and then purified through Percoll™ gradients will die from apoptosis very rapidly if they are left in culture without further stimuli. However, they could be rescued from apoptosis with monoclonal antibody anti-p42 (NIM-R5). Small dense B cells were activated with anti-μ monoclonal antibody (B7.6, 20 μg/ml) plus IL-4 (10 units/ml) for 3 days, and the resulting B cell blasts were purified in a Percoll™ gradient. The cells were incubated again in medium or medium plus IL-4 with or without NIM-R5 for 18 hours. After 4 hours' pulse-labeling with ^{3}H-thymidine, the cells were harvested and the ^{3}H-thymidine incorporated was measured. NIM-R5

induces a small but significant proliferation of these B cell blasts. The proliferation using the antibody alone was weak compared with the proliferation induced in combination with IL-4. IL-4 was also mitogenic for these cells; however, the combination of both stimuli was much higher than the arithmetic addition of the separated factors.

Alternatively, some cells not pulse-labeled with ^{3}H-thymidine were harvested and the total DNA extracted, and analyzed in 1.5% agarose gel containing ethidium bromide. Analysis of the DNA clearly showed the characteristic pattern of degradation seen in the apoptotic phenomena when the cells were cultured, washed, and incubated without further stimulation. However, there was some protection when NIM-R5 was added to the culture. IL-4 also offered protection to these cells. Again the highest protection from apoptosis was seen with the combination of NIM-R5 and IL-4.

Protection from apoptosis also can be indicated by trypan blue dye exclusion with the following results: less than 2% recovery of viable cells after overnight culture in medium alone; approximately 20% viability after culture with NIM-R5; 60% of viable cells with IL-4; and nearly 90% recovery of viable cells with the combination of NIM-R5 plus IL-4.

EXAMPLE 7: <u>NIM-R5 induces the proliferation of LPS activated B cell blasts and this proliferation is synergized or antagonized in a time-dependent fashion by IL-4</u>

B cell blasts (3 days LPS) were induced to proliferate with the monoclonal antibody anti-p42 (NIM-R5). Small dense B cells were stimulated for 3 days with LPS. The resulting B blasts were purified in Percoll™ and recultured again with IL-4 with or without monoclonal antibody NIM-R5. After 4 hours' pulse-labeling with ^{3}H-thymidine, the cells were harvested.

This showed that NIM-R5 induces the proliferation of B cells stimulated for three days with LPS (50 μg/ml). However, in contrast with B cells activated with anti-μ plus IL-4, as described above, in these experiments it was not easy to observe the induction of apoptosis after washing and reculturing the cells. This could be due to the difficulty of eliminating LPS attached to the cells. Thus the Percoll™-purified LPS B cell blasts show some proliferation even without addition of further stimulants, perhaps due to residual LPS. However, the proliferation increases with the addition of NIM-R5.

In contrast to the anti-μ plus IL-4 activated B cell blasts, however, there was no further stimulation through the addition of IL-4 alone, but a dramatic costimulatory effect with the addition of NIM-R5 plus IL-4 was observed at 24

hours' further stimulation. The kinetics of this stimulation are very different if the proliferation is analyzed 24, 72, or 120 hours after washing and the addition of the stimuli. After 24 hours there is a clear costimulatory effect between NIM-R5 and IL-4; in contrast, 72 and 120 hours later not only is this costimulatory effect lost but also there is a clear antagonistic effect. IL-4 clearly reduces the proliferation induced by NIM-R5. The antagonism is not due to reduction in viability, but seems to be more related to the induction of differentiation induced by IL-4.

EXAMPLE 8: NIM-R5 does not recognize CD23, CD40, or CD72.

NIM-R5 recognizes a 42 kD antigen expressed mainly on B cells that is increased upon activation. Because NIM-R5 has been shown to stimulate both resting and activated murine B cells, and because the molecular weight is similar to some other molecules described on B cells, a comparative study was performed to find out if NIM-R5 recognizes CD23, CD40, or CD72. The comparison with these molecules was highly appropriate because: they are cell antigens involved in activation and differentiation of B cells; their molecular weights are similar to p42's; and finally, reagents were not available against these mouse homologues of human CD antigens, in contrast with many B cell mouse CD antigens. In the first approach, biotinylated monoclonal anti CD23 (Pharmingen) and biotinylated anti-CD72 were used in competition or cocapping experiments. (Biotinylated anti-CD72 was a gift of Dr. B. Subbarao, Kentucky University; see Subbarao et al., (1983) *J. Immunol.*, 130:2033-2037.) Binding of both antibodies to B cells was unaffected by binding of NIM-R5. In a second approach, COS7 or L cells transfected with murine CD23 (with a plasmid kindly provided by Dr. Kevin Moore, DNAX Research Institute, Palo Alto, CA; see also Gollnick et al., (1990) *J. Immunol.*, 144:1974-1982), murine CD40 (with a plasmid generated originally by Dr. E. Clark and modified by Dr. N. Harada, DNAX; see Torres et al., (1992) *J. Immunol.*, 148:620-626) and murine CD72-transfected L cells (kind gift of Dr. Jane Parnes, Stanford University; see Nakayama et al., (1989) *Proc. Nat'l Acad. Sci. USA*, 86:1352-1356) were used. Although the three transfectants expressed the CD antigens, none were stained with monoclonal antibody NIM-R5, which must therefore recognize another, as yet undefined, B cell surface antigen.

EXAMPLE 9: cDNA isolation

In order to isolate a cDNA clone encoding the protein recognized by NIM-R5 antibody, a size-selected cDNA library prepared from the mouse pre-B

cell line WEHI 231 was transfected into COS7 cells by electroporation. COS7 cells were harvested 3 days after transfection, and panned directly onto dishes coated with HPLC-purified NIM-R5 monoclonal antibody. Nonadherent cells were washed off, COS7 cells were lysed, and plasmids were recovered and then transformed into *E. coli* for amplification. After the fourth panning, two out of thirty-two plasmids tested were positive for NIM-R5 epitope expression by FACS analysis on COS7 transfectants. Importantly, COS7 cells transfected with either of these two clones did not bind an isotype control anti-CD23 antibody, and NIM-R5 antibody did not bind COS7 cells transfected with an unrelated plasmid. The two cDNA clones encoding the NIM-R5 epitope both contained an insert of 1900 bp, and one clone, called I-19, was chosen for further analysis. Stable transfectants expressing I-19 cDNA were obtained by inserting this clone into a vector containing the Neo resistance gene, and transfecting this plasmid into L cells. Stable transfectants were stained with NIM-R5 antibody or anti-CD23 antibody as an isotype control, then counterstained with phycoerythrin-conjugated streptavidin. Washed cells were analyzed on a FACScan. Following drug selection, the L cell transfectants were strongly positive for NIM-R5 epitope expression by FACS analysis, but did not bind an isotype control antibody. Importantly, NIM-R5 antibody showed no binding to untransfected L cells (see Example 11).

EXAMPLE 10: Characterization of I-19 cDNA

DNA sequence analysis of I-19 cDNA revealed that the 1644 bp insert contained a short 5' untranslated region, an open reading frame of 914 bp, and a 724 bp 3' untranslated region without a poly-A tail (see SEQ ID NO: 1). The open reading frame encoded a polypeptide of 304 amino acids with a predicted molecular mass of 34,500. A hydropathy plot of the deduced amino acid sequence showed a hydrophobic region of 22 amino acids immediately adjacent to 23 amino acids at the amino terminal. This configuration is consistent with the typical features of a type-II transmembrane glycoprotein, where the N terminus of the protein is intracellular. The extracellular domain was composed of 259 amino acids, and contained 7 cysteines and 4 potential N-linked glycosylation sites.

Comparison of both nucleotide and amino acid sequences of I-19 cDNA with GenBank indicated that this cDNA encoded a novel murine protein. However, the search revealed significant homology (73% at the nucleotide level; 70% at the amino acid level) between the I-19 sequence and that of human CD38 (compare SEQ ID NO: 1 and 3). This homology stretched over

the entire cDNA and included marked conservation of cysteine positions. Specifically, all 13 cysteine residues in the I-19 ORF are conserved within the human CD38 sequence. Human CD38 has one additional cysteine residue, located in its short cytoplasmic tail, which is not shared by the I-19 sequence. These data suggest that I-19 cDNA encodes a protein which may be the mouse counterpart of human CD38. Human CD38 is a type-II transmembrane glycoprotein of unknown function which was initially defined by specific monoclonal antibodies as a human lymphocyte activation marker; see, *e.g.*, Reinherz et al., (1980) *Proc. Nat'l Acad. Sci. USA*, 77:1588-1592; Kung et al., (1980) *Vox Sang.*, 39:121-127; Janossy et al., (1981) *J. Immunol.*, 126:1608-1613; Bhan et al., (1981) *J. Exp. Med.*, 154:737-749; Stashenko et al., (1981) *Proc. Nat'l Acad. Sci. USA*, 78:3848-3852; Sieff et al., (1982) *Blood*, 60:703-713; Tedder et al., (1984) *Tissue Antigens*, 24:140-149; and Jackson et al., (1990) *J. Immunol.*, 144:2811-2815.

EXAMPLE 11: I-19 cDNA Encodes the B Cell Activation Marker Recognized by NIM-R5 Antibody

Example 9 demonstrates that I-19 cDNA encodes a B cell derived recombinant protein containing the epitope sequence recognized by NIM-R5 antibody. The following experiments were conducted to evaluate whether this recombinant protein indeed corresponded to the novel B cell activation marker that is activated by NIM-R5 antibody. L cells transfected with I-19 cDNA and untransfected L cells were radio-iodinated on their cell surfaces and then immunoprecipitated with NIM-R5 antibody or an isotype control antibody plus goat anti-rat Ig-Sepharose-4B. NIM-R5 antibody specifically immunoprecipitated a single major band of approximately 45 kd by SDS-PAGE analysis. Thus the size of the I-19 encoded recombinant protein closely resembled that (i.e. 42 kd) of the previously identified activation antigen recognized on normal B lymphocytes by NIM-R5 antibody. The immunoprecipitated recombinant protein was considerably larger than the predicted molecular weight derived from the cDNA sequence, indicating that the molecule is likely to be glycosylated.

The strong similarity between the I-19 cDNA encoded recombinant molecule and the normal B cell activation marker recognized by NIM-R5 was further extended by Northern analyses of I-19 expression. The NIM-R5 epitope was expressed by most normal B cells and B lymphomas and by EL4 thymoma cells, but was not expressed by numerous other cell types including one B lymphoma designated A.20 (see Table 1). mRNA transcripts hybridizing with

the I-19 cDNA probe were expressed by several B lymphomas (*e.g.* CH31, CH12, WEHI 231) and EL4 thymoma cells, but not by A.20 B lymphoma cells or an antigen-activated T cell clone. The results of Examples 9 and 11, collectively indicate that I-19 cDNA encodes a glycoprotein that is indistinguishable biochemically and in terms of cellular distribution from the murine B cell activation marker recognized by NIM-R5 antibody.

EXAMPLE 12: Chromosomal Mapping of Putative Murine CD38 Gene

The mouse chromosomal location of Cd38-rs was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J x Mus spretus)F_1 X C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 1100 loci that are well distributed among all the autosomes as well as the X-chromosome. C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using a mouse cDNA Cd38-rs probe. The 8.4, 5.6, 2.6, and 1.2 kb *M. spretus PstI* RFLPs were used to follow the segregation of the Cd38-rs locus in backcross mice. The mapping results indicated that Cd38-rs is located in the proximal region of mouse chromosome-5 linked to IL-6, Hox-7.1, and Kit. Although 174 mice were analyzed for every marker, up to 203 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombination chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere – IL-6 – 7/203 – Hox-7.1 – 6/176 – Cd38-rs – 26/175 – Kit. The recombination frequencies [expressed as genetic distances in centiMorgans (cM) ± the standard error] are – IL-6 – 3.5 ± 1.3 – Hox-7.1 – 3.4 ± 1.4 – Cd38-rs – 14.9 ± 2.7 - Kit.

In a comparison of the interspecific map of chromosome-5 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (compiled by M.T. Davisson, T.H. Roderick, A.L. Hillyard, and D.P. Doolittle and provided from GBASE, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME), Cd38-rs mapped in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in the Cd38-rs locus.

EXAMPLE 13: Tissue distribution of CD38

Tissue distribution of CD38 was determined by FACS analysis using fluorescently labeled NIM-R5 or α-HEL; see, *e.g.*, Shapiro, (1988) Practical Flow Cytometry (2d ed.), Liss, New York. The results are presented in Table 2:

Table 2: Comparative Cellular Distribution of human and murine CD38.

B Cell Type	Human CD 38	Murine CD 38
Stem	ND	high
pro B	ND	high
pre B	high	high
B	low	high
B blast	low	high
plasma cell	high	ND
germinal center B	high	low

ND = not determined.

This Table shows the comparative cellular distributions of murine and human CD38. Although distribution of the antigen differs between the species, significant similarities exist which are sufficient to suggest similar biological or physiological functions.

EXAMPLE 14: xid B cells are unresponsive to triggering via CD38

Purified B cells from BALB/xid mice or from normal BALB/C mice were stimulated *in vitro* with anti-CD60 or anti-CD38 antibodies in the presence of 100 U/ml of IL-4. The highest concentration of anti-CD40 was a 1/2000 dilution of antiserum. B cells from both the mutant and wild type strains of mice proliferate in response to anti-CD40, but BALB/xid cells do not proliferate in response to anti-CD38. Although BALB/xid B cells do not proliferate in response to anti-CD38, their expression of this molecule appears to be normal.

EXAMPLE 15: Anergic B cells from double transgenic B-tolerant mice are unresponsive to triggering via CD38

Nontransgenic, single transgenic, double transgenic spleen cells, before or after T cell depletion, were stimulated for 48 hours with a titration of α-CD38 antibody, i.e., NIM-R5. The single and double transgenic mice make Ig against hen egg lysozyme (HEL). Cells were plated in 96 well plates at 10^5 cells/well. Proliferation was measured by incorporation of ^{3}H-thymidine over the 48 hours following stimulation. The antibody used to stimulate was titrated over the

range from 500 µg/ml to 1 µg/ml. The non-transgenic and single transgenic mice responded, though the single responded less. The double transgenic mice did not respond.

EXAMPLE 16: Preparation of a soluble CD38 construct

A recombinant construct is made of a soluble CD38 extracellular domain fused to a FLAG sequence used for purification or detection. The construct was made by splicing the extracellular 259-amino-acid coding region of the native murine CD38 onto a signal sequence adjacent to an 8-amino-acid marker (or "FLAG") sequence. The recombinant construct was expressed and purified from Baculovirus-infected insect cells, murine L-cells, and murine COS7 cells.

EXAMPLE 17: Soluble CD38 has an ADP-ribosyl cyclase enzymatic activity

Two separate methods were used to determine cyclase activity. A sea-urchin-egg homogenate calcium flux assay was derived from that reported by Clapper et al., (1987) *J. Biol. Chem.* 262:9561-9568. Alternatively, enzyme reactants and products were analyzed by HPLC using an ion-exchange column, *e.g.*, an AG MP-1 column (Biorad, Richmond, CA).

EXAMPLE 18: Soluble CD38 has an ADP-ribosyl hydrolase enzymatic activity

Two separate methods were used to determine hydrolase activity. Enzymatic reactants and products that were purified by HPLC on an ion-exchange column, *e.g.*, an AG MP-1 column (Biorad, Richmond, CA), showed this activity. Enzymatic metabolites labeled with ^{32}P-NAD and purified by thin-layer chromatography also showed this activity.

EXAMPLE 19: Soluble CD38 has an ADP-ribosyl transferase enzymatic activity

Two separate methods were used to determine transferase activity. Mass spectroscopy of various substrate target proteins incubated with NAD and CD38 showed molecular-weight changes consistent with transfer of ADP-ribose moieties to the proteins. PAGE analysis of these same proteins incubated in the presence of ^{32}P-NAD and CD38 confirmed this enzymatic activity.

EXAMPLE 20: Use of soluble CD38 to screen for pharmacological modulators of the enzyme activity

A monoclonal antibody which recognizes the "flag" sequence of the recombinant CD38-FLAG fusion construct is attached to a solid substrate. The CD38-FLAG fusion protein is added and attaches to the substrate via the antibody. The FLAG attachment is so designed that it does not significantly interfere with enzymatic activity of the protein. The enzyme substrate NAD is added to the solid phase attached enzyme. NAD is converted into cADPR and ADP-ribose. The resulting reaction supernatant is run on HPLC to detect either substrate or product. A time course or final point may be assayed. The assay can be simplified with the use of radio-labeled NAD and separating the NAD, cADPR and ADPR by thin layer chromatography. Other activities, *e.g.*, hydrolase activity or transferase activity, can also be used.

The assay may also be used to screen for modulators of enzymatic activity. Various candidate compounds may be tested for an effect on an enzymatic activity, with the expectation that the enzyme activity is critical in the immunological function dependent upon CD38. More particularly, this assay can be used to screen for potential compounds which block the CD38 cyclase, hydrolase, and/or transferase activities. Potential blocking compounds could be added prior to the addition of NAD. Compounds which block the activity would be identified. An enzyme blocking analog could in turn be used as a potential drug to block or alter the biological effects of CD38. Attractive candidates for such screening include NAD analogs.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Schering Corporation

(B) STREET: 2000 Galloping Hill Road

(C) CITY: Kenilworth

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) POSTAL CODE: 07033

(i) APPLICANT:

(A) NAME: The Board of Trustees of The Leland Stanford Junior University

(B) STREET: 900 Welch Road, Suite 350

(C) CITY: Palo Alto

(D) STATE: California

(E) COUNTRY: USA

(F) POSTAL CODE: 94304-1850

(i) APPLICANT:

(A) NAME: R. Michael E. Parkhouse

(B) STREET: 7 BullsWater Crescent Road [off Ash Road]

(C) CITY: Purbright, Woking

(D) COUNTY: Surrey

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: GU240NF

(ii) TITLE OF INVENTION: MODULATION OF PHYSIOLOGICAL RESPONSES OF LYMPHOCYTES

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: John H. C. Blasdale

(B) COMPANY: Schering-Plough Corporation

(C) STREET: One Giralda Farms

(D) CITY: Madison

(E) STATE: New Jersey

(F) COUNTRY: USA

(G) ZIP: 07940-1000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Macintosh 6.0.5

(D) SOFTWARE: Microsoft Word 5.1a

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US 94/[remainder unknown

(B) FILING DATE: [herewith]

(C) CLASSIFICATION: [unknown]

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/010,905

(B) FILING DATE: 29-JAN-1993

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Blasdale, John H. C.

(B) REGISTRATION NUMBER: 31,895

(C) REFERENCE/DOCKET NUMBER: DX0351K

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-822-7398

(B) TELEFAX: 201-822-7039

(C) TELEX: 219165

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1644 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 7..921

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
AAGCCA ATG GCT AAC TAT GAA TTT AGC CAG GTG TCT GGG GAC AGA CCT      48
       Met Ala Asn Tyr Glu Phe Ser Gln Val Ser Gly Asp Arg Pro
         1               5                  10

GGC TGC CGC CTC TCT AGG AAA GCC CAG ATC GGT CTC GGA GTG GGT CTC      96
Gly Cys Arg Leu Ser Arg Lys Ala Gln Ile Gly Leu Gly Val Gly Leu
 15                  20                  25                  30

CTG GTC CTG ATC GCC TTG GTA GTA GGG ATC GTG GTC ATA CTT CTG AGG     144
Leu Val Leu Ile Ala Leu Val Val Gly Ile Val Val Ile Leu Leu Arg
                 35                  40                  45

CCG CGC TCA CTC CTG GTG TGG ACT GGA GAG CCT ACC ACG AAG CAC TTT     192
Pro Arg Ser Leu Leu Val Trp Thr Gly Glu Pro Thr Thr Lys His Phe
             50                  55                  60

TCT GAC ATC TTC CTG GGA CGC TGC CTC ATC TAC ACT CAG ATC CTC CGG     240
Ser Asp Ile Phe Leu Gly Arg Cys Leu Ile Tyr Thr Gln Ile Leu Arg
         65                  70                  75

CCG GAG ATG AGA GAT CAG AAC TGC CAG GAG ATA CTG AGT ACA TTC AAA     288
Pro Glu Met Arg Asp Gln Asn Cys Gln Glu Ile Leu Ser Thr Phe Lys
     80                  85                  90

GGA GCA TTT GTT TCC AAG AAC CCT TGC AAC ATC ACA AGA GAA GAC TAC     336
Gly Ala Phe Val Ser Lys Asn Pro Cys Asn Ile Thr Arg Glu Asp Tyr
 95                  100                 105                 110

GCC CCA CTT GTT AAA TTG GTC ACT CAA ACC ATA CCA TGT AAC AAG ACT     384
Ala Pro Leu Val Lys Leu Val Thr Gln Thr Ile Pro Cys Asn Lys Thr
                115                 120                 125
```

```
CTC TTT TGG AGC AAA TCC AAA CAC CTG GCC CAT CAA TAT ACT TGG ATC    432
Leu Phe Trp Ser Lys Ser Lys His Leu Ala His Gln Tyr Thr Trp Ile
            130                 135                 140

CAG GGA AAG ATG TTC ACC CTG GAG GAC ACC CTG CTG GGC TAC ATT GCT    480
Gln Gly Lys Met Phe Thr Leu Glu Asp Thr Leu Leu Gly Tyr Ile Ala
        145                 150                 155

GAT GAT CTC AGG TGG TGT GGA GAC CCT AGT ACT TCT GAT ATG AAC TAT    528
Asp Asp Leu Arg Trp Cys Gly Asp Pro Ser Thr Ser Asp Met Asn Tyr
    160                 165                 170

GTC TCT TGC CCA CAT TGG AGT GAA AAC TGT CCC AAC AAC CCT ATT ACT    576
Val Ser Cys Pro His Trp Ser Glu Asn Cys Pro Asn Asn Pro Ile Thr
175                 180                 185                 190

ATG TTC TGG AAA GTG ATT TCC CAA AAG TTT GCA GAA GAT GCC TGT GGT    624
Met Phe Trp Lys Val Ile Ser Gln Lys Phe Ala Glu Asp Ala Cys Gly
                195                 200                 205

GTG GTC CAA GTG ATG CTC AAT GGG TCC CTC CGT GAG CCG TTT TAC AAA    672
Val Val Gln Val Met Leu Asn Gly Ser Leu Arg Glu Pro Phe Tyr Lys
            210                 215                 220

AAC AGC ACC TTT GGA AGT TTG GAA GTC TTT AGT TTG GAC CCA AAT AAG    720
Asn Ser Thr Phe Gly Ser Leu Glu Val Phe Ser Leu Asp Pro Asn Lys
        225                 230                 235

GTT CAT AAA CTA CAG GCC TGG GTG ATG CAC GAC ATC GAA GGA GCT TCC    768
Val His Lys Leu Gln Ala Trp Val Met His Asp Ile Glu Gly Ala Ser
    240                 245                 250

AGT AAC GCA TGT TCA AGC TCC TCC TTA AAT GAG CTG AAG ATG ATT GTG    816
Ser Asn Ala Cys Ser Ser Ser Ser Leu Asn Glu Leu Lys Met Ile Val
255                 260                 265                 270

CAG AAA AGG AAT ATG ATA TTT GCC TGC GTG GAT AAC TAC AGG CCT GCC    864
Gln Lys Arg Asn Met Ile Phe Ala Cys Val Asp Asn Tyr Arg Pro Ala
                275                 280                 285

AGG TTT CTT CAG TGT GTG AAG AAC CCT GAG CAC CCA TCG TGT AGA CTT    912
Arg Phe Leu Gln Cys Val Lys Asn Pro Glu His Pro Ser Cys Arg Leu
            290                 295                 300

AAT ACG TGAAGGATCT GGATCTTAGA TCACCTGTAG CCTGGACTGA GATGAAGGGG     968
Asn Thr
        305

CTCAGAAGCA ACACTGGTGG AAAGCTGAAA CTGTCAGGGA GAAGCCTCTA CTACAGTGTT 1028

AACACCAGAG ATGGAAGAAC TTCCCAATTC TCTGTGTACT ACCAACATTC AAGAAAAATT 1088

ACTCCATAAA CCAGAGTTAA ACTTCTATAT TGTTATATTA GTCTAACTTT CTCATGTGGT 1148

GCTTCTGTAT TGTTTATATA TTGCTTACAT CCTTTTATTC CTCTTTTAAT GATCTCTCTT 1208
```

TTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCAATGAGG 1268

CTGAGAATCC AACCTGAGAA CTTTCATACA TGGTGGATAA GCCTATATAC CACTGAGCTA 1328

AATCCTCAGC ACAGCTGATA ACATCATTTT TGCTGAAAAA TGGCCAATCA AACTTCCCAT 1388

TAGACAAAGA AAGTCAAATG TCAAGTATAT CGAAATGAAT GACCCTTTTT TTTTTATGTT 1448

TTTTCATTCT TCCCACAGAT ATTCACATGG TAAACCTGAG GTCATAGGGT CATTATAGGG 1508

AAGGTGCTGT GTGGGAACTA CCCACGTGCC CTGTGCTTTA ATCTTTAACT CAACACGTCC 1568

CTGATAACTT TGAGCATTCT TTTCTTTTCT TTTCTTTTCT TTTCTTTTCT TTTCTTTTCT 1628

TTTTCTTTTT TTCTTT 1644

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Asn Tyr Glu Phe Ser Gln Val Ser Gly Asp Arg Pro Gly Cys
1 5 10 15

Arg Leu Ser Arg Lys Ala Gln Ile Gly Leu Gly Val Gly Leu Leu Val
20 25 30

Leu Ile Ala Leu Val Val Gly Ile Val Val Ile Leu Leu Arg Pro Arg
35 40 45

Ser Leu Leu Val Trp Thr Gly Glu Pro Thr Thr Lys His Phe Ser Asp
50 55 60

Ile Phe Leu Gly Arg Cys Leu Ile Tyr Thr Gln Ile Leu Arg Pro Glu
65 70 75 80

Met Arg Asp Gln Asn Cys Gln Glu Ile Leu Ser Thr Phe Lys Gly Ala
85 90 95

Phe Val Ser Lys Asn Pro Cys Asn Ile Thr Arg Glu Asp Tyr Ala Pro
100 105 110

Leu Val Lys Leu Val Thr Gln Thr Ile Pro Cys Asn Lys Thr Leu Phe
115 120 125

Trp Ser Lys Ser Lys His Leu Ala His Gln Tyr Thr Trp Ile Gln Gly
130 135 140

Lys Met Phe Thr Leu Glu Asp Thr Leu Leu Gly Tyr Ile Ala Asp Asp
145 150 155 160

Leu Arg Trp Cys Gly Asp Pro Ser Thr Ser Asp Met Asn Tyr Val Ser
165 170 175

Cys Pro His Trp Ser Glu Asn Cys Pro Asn Asn Pro Ile Thr Met Phe
180 185 190

Trp Lys Val Ile Ser Gln Lys Phe Ala Glu Asp Ala Cys Gly Val Val
195 200 205

Gln Val Met Leu Asn Gly Ser Leu Arg Glu Pro Phe Tyr Lys Asn Ser
210 215 220

Thr Phe Gly Ser Leu Glu Val Phe Ser Leu Asp Pro Asn Lys Val His
225 230 235 240

Lys Leu Gln Ala Trp Val Met His Asp Ile Glu Gly Ala Ser Ser Asn
245 250 255

Ala Cys Ser Ser Ser Ser Leu Asn Glu Leu Lys Met Ile Val Gln Lys
260 265 270

Arg Asn Met Ile Phe Ala Cys Val Asp Asn Tyr Arg Pro Ala Arg Phe
275 280 285

Leu Gln Cys Val Lys Asn Pro Glu His Pro Ser Cys Arg Leu Asn Thr
290 295 300

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1407 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 70..972

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CTAAAGCTCT CTTGCTGCCT AGCCTCCTGC CGGCCTCATC TTCGCCCAGC CAACCCCGCC   60

TGGAGCCCT ATG GCC AAC TGC GAG TTC AGC CCG GTG TCC GGG GAC AAA       108
          Met Ala Asn Cys Glu Phe Ser Pro Val Ser Gly Asp Lys
            1               5                  10

CCC TGC TGC CGG CTC TCT AGG AGA GCC CAA CTC TGT CTT GGC GTC AGT     156
Pro Cys Cys Arg Leu Ser Arg Arg Ala Gln Leu Cys Leu Gly Val Ser
     15                  20                  25

ATC CTG GTC CTG ATC CTC GTC GTG GTG CTC GCG GTG GTC GTC CCG AGG     204
Ile Leu Val Leu Ile Leu Val Val Val Leu Ala Val Val Val Pro Arg
 30                  35                  40                  45

TGG CGC CAG ACG TGG AGC GGT CCG GGC ACC ACC AAG CGC TTT CCC GAG     252
Trp Arg Gln Thr Trp Ser Gly Pro Gly Thr Thr Lys Arg Phe Pro Glu
                 50                  55                  60

ACC GTC CTG GCG CGA TGC GTC AAG TAC ACT GAA ATT CAT CCT GAG ATG     300
Thr Val Leu Ala Arg Cys Val Lys Tyr Thr Glu Ile His Pro Glu Met
           65                  70                  75

AGA CAT GTA GAC TGC CAA AGT GTA TGG GAT GCT TTC AAG GGT GCA TTT     348
Arg His Val Asp Cys Gln Ser Val Trp Asp Ala Phe Lys Gly Ala Phe
      80                  85                  90

ATT TCA AAA CAT CCT TGC AAC ATT ACT GAA GAA GAC TAT CAG CCA CTA     396
Ile Ser Lys His Pro Cys Asn Ile Thr Glu Glu Asp Tyr Gln Pro Leu
 95                  100                 105

ATG AAG TTG GGA ACT CAG ACC GTA CCT TGC AAC AAG ATT CTT CTT TGG     444
Met Lys Leu Gly Thr Gln Thr Val Pro Cys Asn Lys Ile Leu Leu Trp
110                 115                 120                 125

AGC AGA ATA AAA GAT CTG GCC CAT CAG TTC ACA CAG GTC CAG CGG GAC     492
Ser Arg Ile Lys Asp Leu Ala His Gln Phe Thr Gln Val Gln Arg Asp
                130                 135                 140

ATG TTC ACC CTG GAG GAC ACG CTG CTA GGC TAC CTT GCT GAT GAC CTC     540
Met Phe Thr Leu Glu Asp Thr Leu Leu Gly Tyr Leu Ala Asp Asp Leu
           145                 150                 155

ACA TGG TGT GGT GAA TTC AAC ACT TCC AAA ATA AAC TAT CAA TCT TGC     588
Thr Trp Cys Gly Glu Phe Asn Thr Ser Lys Ile Asn Tyr Gln Ser Cys
      160                 165                 170

CCA GAC TGG AGA AAG GAC TGC AGC AAC AAC CCT GTT TCA GTA TTC TGG     636
Pro Asp Trp Arg Lys Asp Cys Ser Asn Asn Pro Val Ser Val Phe Trp
 175                 180                 185

AAA ACG GTT TCC CGC AGG TTT GCA GAA GCT GCC TGT GAT GTG GTC CAT     684
Lys Thr Val Ser Arg Arg Phe Ala Glu Ala Ala Cys Asp Val Val His
190                 195                 200                 205
```

GTG ATG CTC AAT GGA TCC CGC AGT AAA ATC TTT GAC AAA AAC AGC ACT 732
Val Met Leu Asn Gly Ser Arg Ser Lys Ile Phe Asp Lys Asn Ser Thr
210 215 220

TTT GGG AGT GTG GAA GTC CAT AAT TTG CAA CCA GAG AAG GTT CAG ACA 780
Phe Gly Ser Val Glu Val His Asn Leu Gln Pro Glu Lys Val Gln Thr
225 230 235

CTA GAG GCC TGG GTG ATA CAT GGT GGA AGA GAA GAT TCC AGA GAC TTA 828
Leu Glu Ala Trp Val Ile His Gly Gly Arg Glu Asp Ser Arg Asp Leu
240 245 250

TGC CAG GAT CCC ACC ATA AAA GAG CTG GAA TCG ATT ATA AGC AAA AGG 876
Cys Gln Asp Pro Thr Ile Lys Glu Leu Glu Ser Ile Ile Ser Lys Arg
255 260 265

AAT ATT CAA TTT TCC TGC AAG AAT ATC TAC AGA CCT GAC AAG TTT CTT 924
Asn Ile Gln Phe Ser Cys Lys Asn Ile Tyr Arg Pro Asp Lys Phe Leu
270 275 280 285

CAG TGT GTG AAA AAT CCT GAG GAT TCA TCT TGC ACA TCT GAG ATC 969
Gln Cys Val Lys Asn Pro Glu Asp Ser Ser Cys Thr Ser Glu Ile
290 295 300

TGAGCCAGTC GCTGTGGTTG TTTTAGCTCC TTGACTCCTT GTGGTTTATG TCATCATACA 1029

TGACTCAGCA TACCTGCTGG TGCAGAGCTG AAGATTTTGG AGGGTCCTCC ACAATAAGGT 1089

CAATGCCAGA GACGGAAGCC TTTTTCCCCA AAGTCTTAAA ATAACTTATA TCATCAGCAT 1149

ACCTTTATTG TGATCTATCA ATAGTCAAGA AAAATTATTG TATAAGATTA GAATGAAAAT 1209

TGTATGTTAA GTTACTTCCT TTAGAGCACA ATGGATCTCG AGGGATCTTC CATACCTACC 1269

AGTTCTGCGC CTGCGAGTCG CGGCCGCATC TAGAGGATCT TTGTGAAGGA ACCTTACTTC 1329

TGTGGTGTGA CATAATTGGA CAAACTACCT ATAGAGATTT AAAGCTCTAA GGTAAATATA 1389

AAATTTTTAA GTGTATAA 1407

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 300 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Asn Cys Glu Phe Ser Pro Val Ser Gly Asp Lys Pro Cys Cys
 1               5                  10                  15

Arg Leu Ser Arg Arg Ala Gln Leu Cys Leu Gly Val Ser Ile Leu Val
            20                  25                  30

Leu Ile Leu Val Val Val Leu Ala Val Val Val Pro Arg Trp Arg Gln
        35                  40                  45

Thr Trp Ser Gly Pro Gly Thr Thr Lys Arg Phe Pro Glu Thr Val Leu
    50                  55                  60

Ala Arg Cys Val Lys Tyr Thr Glu Ile His Pro Glu Met Arg His Val
65                  70                  75                  80

Asp Cys Gln Ser Val Trp Asp Ala Phe Lys Gly Ala Phe Ile Ser Lys
                85                  90                  95

His Pro Cys Asn Ile Thr Glu Glu Asp Tyr Gln Pro Leu Met Lys Leu
            100                 105                 110

Gly Thr Gln Thr Val Pro Cys Asn Lys Ile Leu Leu Trp Ser Arg Ile
        115                 120                 125

Lys Asp Leu Ala His Gln Phe Thr Gln Val Gln Arg Asp Met Phe Thr
    130                 135                 140

Leu Glu Asp Thr Leu Leu Gly Tyr Leu Ala Asp Asp Leu Thr Trp Cys
145                 150                 155                 160

Gly Glu Phe Asn Thr Ser Lys Ile Asn Tyr Gln Ser Cys Pro Asp Trp
                165                 170                 175

Arg Lys Asp Cys Ser Asn Asn Pro Val Ser Val Phe Trp Lys Thr Val
            180                 185                 190

Ser Arg Arg Phe Ala Glu Ala Ala Cys Asp Val Val His Val Met Leu
        195                 200                 205

Asn Gly Ser Arg Ser Lys Ile Phe Asp Lys Asn Ser Thr Phe Gly Ser
    210                 215                 220

Val Glu Val His Asn Leu Gln Pro Glu Lys Val Gln Thr Leu Glu Ala
225                 230                 235                 240

Trp Val Ile His Gly Gly Arg Glu Asp Ser Arg Asp Leu Cys Gln Asp
                245                 250                 255

Pro Thr Ile Lys Glu Leu Glu Ser Ile Ile Ser Lys Arg Asn Ile Gln
            260                 265                 270

Phe Ser Cys Lys Asn Ile Tyr Arg Pro Asp Lys Phe Leu Gln Cys Val
        275                 280                 285

Lys Asn Pro Glu Asp Ser Ser Cys Thr Ser Glu Ile
    290                 295                 300
```

CLAIMS:

1. A method of modulating a physiological response of a lymphocyte comprising contacting said lymphocyte with:

a) an antibody to CD38;

b) a soluble fragment of CD38; or

c) a pharmacological modulator of ADP-ribosyl cyclase or cyclic ADP-ribosyl hydrolase.

2. A method of Claim 1, wherein said modulating is selected from stimulation or inhibition of lymphocyte growth or differentiation.

3. A method of Claim 1, wherein said modulating is stimulation of lymphocyte differentiation.

4. A method of Claim 3, wherein said differentiation results in establishment of antigen tolerance.

5. A method of Claim 1, wherein said physiological response is mediated by a calcium flux.

6. A method of Claim 1, wherein said lymphocyte is a B cell.

7. A method of Claim 1, wherein said lymphocyte is at a defined developmental stage.

8. A method of Claim 7, wherein said developmental stage expresses surface CD38.

9. A method of Claim 1, wherein said antibody to CD38 is polyclonal.

10. A method of Claim 1, wherein said antibody to CD38 is NIMR5.

11. A method of Claim 1, wherein said soluble fragment of CD38 consists essentially of the extracellular region of CD38.

12. A method of Claim 1, wherein said pharmacological modulator is an inhibitor of ADP-ribosyl cyclase.

13. A method of modulating an antigen tolerance response of a B lymphocyte comprising contacting said lymphocyte with:

a) an antibody to CD38;
b) a soluble fragment of CD38;
c) a pharmacological modulator of ADP-ribosyl cyclase; or
d) a pharmacological modulator of cyclic ADP-ribosyl hydrolase.

14. A method of Claim 13, wherein said modulating is inducing said antigen tolerance response.

15. A method of Claim 13, wherein said antibody to CD38 is NIM-R5.

16. A method of screening for a pharmacological modulator of ADP-ribosyl cyclase activity or cyclic ADP-ribosyl hydrolase activity of CD38, comprising the steps of:

assaying ADP-ribosyl cyclase activity or cyclic ADP-ribosyl hydrolase activity of CD38 in the presence or absence of a candidate pharmacological modulator; and

selecting a candidate which modulates said activity.

17. A method of Claim 16, wherein said enzymatic activity is ADP-ribosyl cyclase activity.

18. A method of Claim 16, wherein said candidate is selected from a group of NAD analogs.

19. A pharmacological modulator selected by a method of Claim 16.

20. A pharmacological modulator of Claim 19 which also modulates a physiological response of a lymphocyte, including a B cell.

21. Murine CD38.

22. An isolated DNA sequence encoding murine CD38.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 94/00517

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C07K13/00 C12P21/08 A61K37/02 A61K39/395
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C12P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 330 191 (THE GENERAL HOSPITAL CORPORATION, US) 30 August 1989 see page 5, line 5 - page 6, line 16 see page 25, line 69 - page 26, line 50; claims 1-10 ---	1-9, 11-14, 16-20
Y	TIBS TRENDS IN BIOCHEMICAL SCIENCES vol. 17 , December 1992 , CAMBRIDGE EN page 495 'Similarities in amino acid sequences of Apysia ADP-ribosyl cyclase and human lymphocyte antigen CD38' see the whole document --- -/--	1-9, 11-14, 16-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 May 1994	15 -06- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nauche, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLOOD vol. 77, no. 5 , 1 March 1991 pages 1071 - 1079 STEVENSON FK;BELL AJ;CUSACK R;HAMBLIN TJ;SLADE CJ;SPELLERBERG MB;STEVENSON GT; 'Preliminary studies for an immunotherapeutic approach to the treatment of human myeloma using chimeric anti-CD38 antibody.' see the whole document	1-3,5-9, 12,13
Y	JOURNAL OF IMMUNOLOGY. vol. 145, no. 3 , 1 August 1990 , BALTIMORE US pages 878 - 884 ALESSIO M;ROGGERO S;FUNARO A;DE MONTE LB;PERUZZI L;GEUNA M;MALAVASI F; 'CD38 molecule: structural and biochemical analysis on human T lymphocytes, thymocytes, and plasma cells.' see the whole document	1-9, 11-14, 16-20
Y	JOURNAL OF IMMUNOLOGY. vol. 144, no. 7 , 1 April 1990 , BALTIMORE US pages 2811 - 2815 JACKSON, D.G. ET AL.; 'Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinous pattern of expression during lymphocyte differentiation.' see the whole document	1-9, 11-14, 16-20
Y	INTERNATIONAL JOURNAL OF CLINICAL AND LABORATORY RESEARCH vol. 22, no. 2 , July 1992 pages 73 - 80 MALAVASI, F. ET AL.; 'CD38: A multi-lineage cell activation with a split personality' see the whole document	1-9, 11-14, 16-20
P,X	THE JOURNAL OF CELLULAR BIOCHEMISTRY vol. 17, no. B , February 1993 page 213 GRIMALDI, J.C. ET AL; 'Production of soluble murine CD38 for studying its biological functions' & Keystone Symposium on molecular aspects of B lymphocyte differentiation, TAOS, New Mexico, US; February 1-8, 1993 See Abstract FZ139	21,22

-/--

2

Int .onal Application No
PCT/US 94/00517

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Week 9134, Derwent Publications Ltd., London, GB; AN 91-250655 & SU,A,1 595 902 (A MED USSR ONCOLOGY) 30 September 1990 see abstract -----	

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 94/00517

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 1-15 are directed to a method of treatment of the human/animal body as well as diagnostic methods, as far as they are applied in vivo, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 94/00517

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0330191	30-08-89	AU-B-	627710	03-09-92
		AU-A-	2947389	22-09-89
		WO-A-	8908114	08-09-89